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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1970

J. D. MacEWEN

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SYSTEMED CORPORATION

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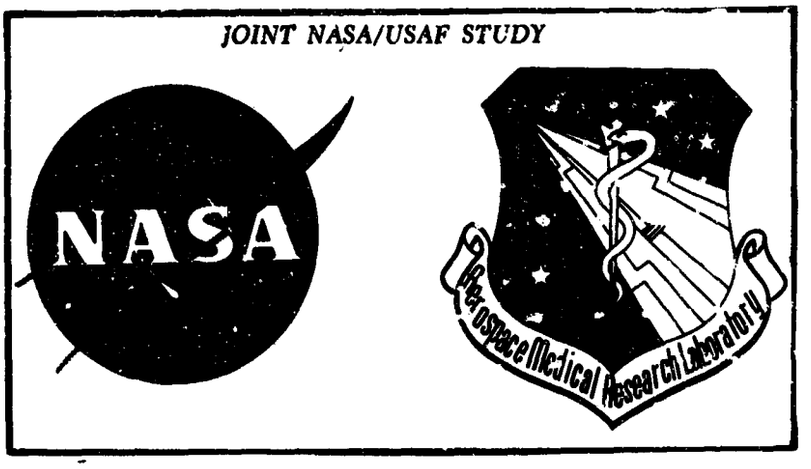

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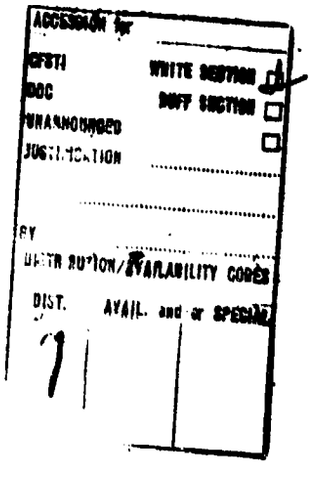

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13. ABSTRACT

The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1969 through May 1970 are reviewed in this report. Modification of the animal exposure facilities are discussed including the installation of an automatic weighing system in each Thomas Dome. Acute toxicity experiments were conducted on beta cloth glass fiber dust, chlorinetrifluoride (ClF_3), oxygen difluoride (OF_2), and hydrogen fluoride. Subacute toxicity studies were conducted on 1,1,2-Trichloro 1,2,2-trifluoroethane and methylisobutylketone. The interim results of chronic toxicity experiments on monomethylhydrazine (MMH) are also described.

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Oxygen Difluoride						
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Monomethylhydrazine						
Methylisobutylketone						
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FOREWORD

This is the sixth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by SysteMed Corporation on behalf of the Air Force under Contracts No. F33615-67-C-1025 and F33615-70-C-1046. This constitutes the first report under the current contract and describes the accomplishments of the THRU from June 1969 through May 1970.

The contract for operation of the laboratory was initiated in 1963 under Project 6302 "Toxic Hazards of Propellants and Materials," Task No. 01 "Toxicology" Work Unit No. 008 and continued under No. 010. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, PhD, of SysteMed Corporation, served as principal investigator and Laboratory Director for the THRU. Acknowledgement is made to C. E. Johnson, C. C. Haun, G. L. Fogle and J. H. Archibald for their significant contributions and assistance in the preparation of this report. The National Aeronautics and Space Administration provided support for Apollo Materials Screening Program.

This report is designated as SysteMed Corporation Report No. W-70005.

This technical report has been reviewed and is approved.

C. H. KRATOCHVIL, Colonel, USAF, MC
Commander
Aerospace Medical Research Laboratory

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SECTION I

INTRODUCTION

The Toxic Hazards Research Unit (THRU) provides toxicologic investigations of potentially hazardous materials of interest to the Air Force. These investigations, conducted by SysteMed Corporation personnel, are designed to characterize the acute or chronic toxic effects of materials to which military or civilian personnel may be accidentally or unavoidably exposed. Considerable research is also conducted for the National Aeronautics and Space Administration to define the toxicological hazards of space flight and to establish safe environmental standards for such flights. The toxicologic research of manned space flight problems is concerned with defining the risk of breathing trace air contaminants resulting from outgassing of agents incorporated in cabin construction materials and from chemicals used for propulsion and life support systems. This research is conducted on several species of laboratory animals under conditions which simulate space flight as closely as possible, with the exception of radiation and weightlessness.

The research operations of the THRU, conducted by SysteMed Corporation personnel, are supported by the Veterinary Medicine Division and the Toxic Hazards Division of the Aerospace Medical Research Laboratories. These support services include veterinary medical care, procurement of laboratory animals, and both clinical and anatomical pathology examinations of animal tissues.

The continuing research programs of the THRU involving the interdisciplinary approach of the inhalation toxicology team (analytical chemistry, medical technology, pathology, engineering and biological sciences) are conducted in a group of laboratories surrounding the animal exposure facilities. These facilities are three types of animal exposure chambers, each performing a separate specialized function. Preconditioning chambers are used to prepare and stabilize animals in a controlled environment. Rochester and Longley Chambers are used for exposing animals to atmospheric contaminants under ambient conditions of pressure and air composition. Two groups of four specially designed altitude chambers (designated hereafter as Thomas Domes) are utilized for similarly exposing animals to atmospheric compositions of 100% oxygen or varying mixtures of oxygen and nitrogen at pressures ranging from ambient to as low as 5 psia (1/3 atmosphere). The Thomas Domes are equally useful for the conduct of chronic toxicity studies at ambient conditions such as the simulation of long duration continuous exposures to low concentrations of air pollutant materials. More detailed discussion on the design and operation of the THRU facility is published in references 10, 29, 30, 31, 33 and 48.

This report summarizes the research accomplishment of the THRU from June 1969 through May 1970 and includes various facility and design modifications made since the last annual report (reference 33). During the past year a Military Construction Program (MCP) for additional research facilities was completed. The new facilities included the second set of four Thomas Dome animal exposure chambers which are equipped with an interconnecting airlock. The interconnecting airlock was specially designed for use as a surgical suite or physiological testing center where experimental animals can be tested under the same environmental conditions as those used in their exposure chamber. The first year of experience with the new Thomas Domes and the interconnecting airlock began with a shakedown period of several toxicity exposure studies. Some engineering design and construction deficiences were uncovered during this period and have been satisfactorily corrected.

SECTION II

FACILITIES

The operation of a research laboratory for conducting applied toxicologic investigations requires a variety of supporting activities in addition to those provided by the Air Force as described earlier. Many of these activities, while important to the primary mission of the THRU, are not of sufficient magnitude to merit separate technical reports and are, therefore, discussed under the general heading of "Facilities." Included herein are special projects in analytical chemistry, training programs, computer program services and special engineering modifications to the research facilities.

The standard operating procedures (SOP's) that had been revised during the previous report period were tested under use conditions with the new Thomas Domes. Three SOP's required further revision due to modification of equipment, its relocation or changes in the control systems. The procedures revised were "Vacuum Pump Failure," "Air Compressor Failure" and "Complete Power Failure."

COMPUTER PROGRAM SERVICES

The computer programs described in the 1968 annual report (reference 31) were modified to analyze data from current experiments. Preexposure animal data were again compiled and subjected to computer analysis to establish the biochemical characteristics of the populations used in our research programs. This reevaluation of the animal population characteristics was made necessary by changes in sources of animal procurement and modifications in clinical laboratory methods.

The computer program services were utilized during the past year in areas of program management, particularly for property control inventory records. The property control system was completely updated and a standardized nomenclature developed. The computerized property control system has simplified some of the administrative aspects of inventory and property protection.

ANALYTICAL CHEMISTRY PROGRAMS

The primary function of the Analytical Chemistry Department of the THRU is to perform the routine tasks of monitoring animal exposure chamber contaminant concentrations, thus assuring the uniformity and reliability of controlled experiments necessary for meaningful interpretation of the measured biological responses. Preceding the regular analysis

of chamber environments is the more challenging task of developing or modifying methods for analysis of the contaminant to be tested. The ultimate goal of method selection for development in the THRU is continuous automatic monitoring.

Many analytical projects, although equally important, do not directly relate to the toxicological research progress. These projects, including contaminant pyrolysis product studies and methods development for related Air Force toxicity experiments, are the subject of this portion of the annual report.

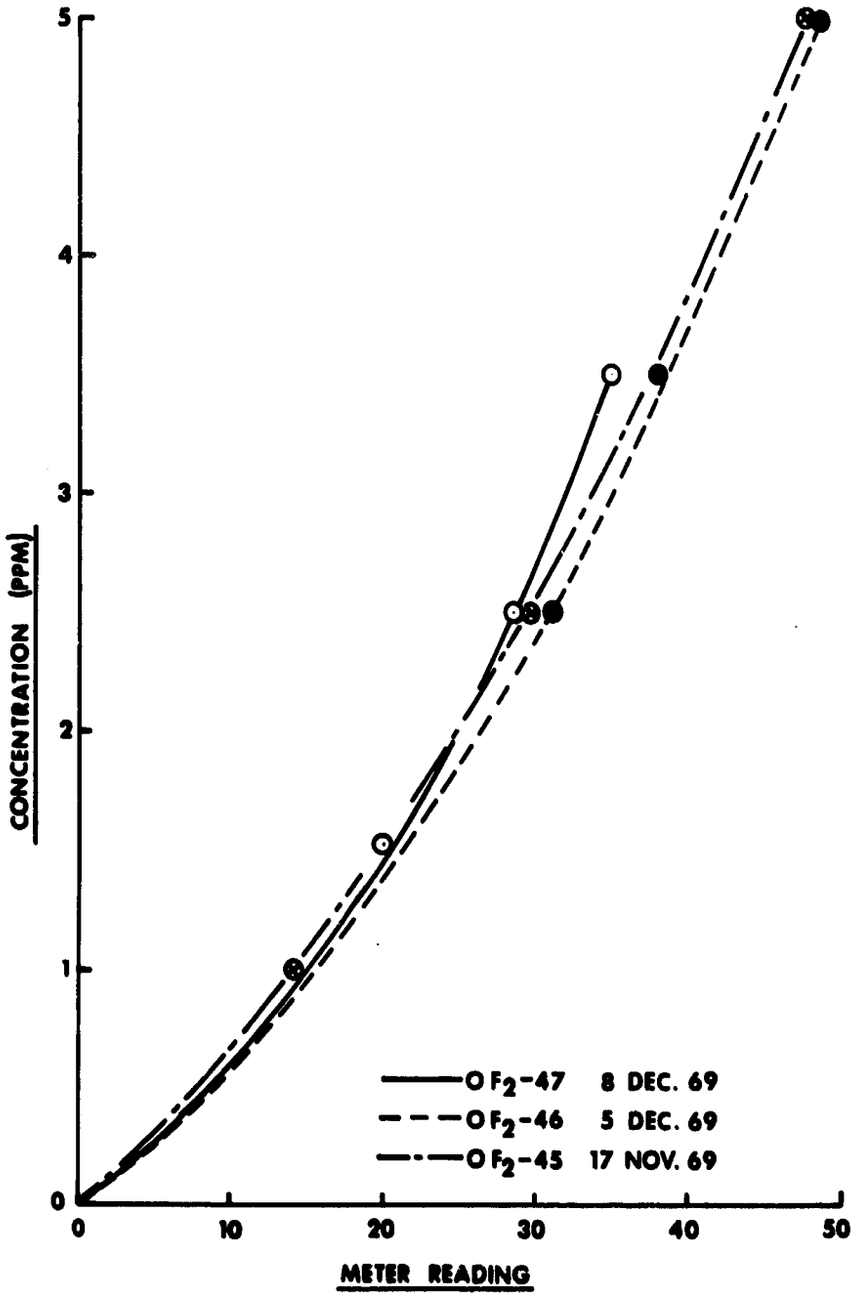
Analysis of Oxygen Difluoride

This compound is probably the most toxic of all the oxidizers of interest to the Air Force, and the investigation of its acute toxicity was scheduled to take place immediately after the termination of chlorine trifluoride experiments. A method was, therefore, developed for the continuous analysis of OF_2 in the 0-5 ppm range which could be expanded to 0-50 ppm by dilution of the sample with dry air. The MSA Billionaire, operating in its most sensitive mode, was found to be satisfactory for the analysis of OF_2 in this range. The OF_2 is pyrolyzed in a unit supplied with the instrument, and the pyrolyzate is reacted with dimethylamine to form an aerosol whose concentration is measured by the Billionaire electron capture detector. Under the conditions selected, the concentration of OF_2 in the chamber air is proportional to the response of the detector. Care must be taken with this procedure since the Billionaire is operating at high sensitivity. An accurate and stable baseline must be obtained before calibration or measurement can be done, and the instrument must be warmed up overnight to preclude baseline drift.

When these precautions are taken, very reproducible data may be obtained from day to day as demonstrated in figure 1 which shows a maximum deviation of 0.4 ppm at about 3.2 ppm for calibration curves run on three different days.

Analysis of Freon 113

For control of exposures of experimental animals to Freon 113 for periods ranging from one hour to two weeks, a gas chromatographic method was developed for the analysis of Freon 113 at concentrations of 500-3000 ppm. Under the conditions of the method, analyses could be repeated approximately every two minutes, which was satisfactory for the monitoring of acute exposures. The method utilized a five-foot Porasil C column operated at a temperature of 100 C and a thermal conductivity detector.



COMPARISON OF OF_2 CALIBRATION DATA TAKEN ON DIFFERENT DAYS

Figure 1

Comparison of OF_2 Calibration Data
Taken on Different Days

Analysis of Chlorine Trifluoride

Initial experiments with ClF_3 had indicated that the toxicity of this compound might be explained by assuming complete hydrolysis of ClF_3 to HF during exposure. Therefore, an infrared technique was devised for measuring ClF_3 concentration over the range 0-1000 ppm. The IR cell utilized was constructed of Teflon® of 10 cm path length with silver chloride windows. The absorbance maximum at 14.25 microns measured on the Beckman IR-5A spectrophotometer was used to determine the ClF_3 concentration based on the Beer-Lambert law. Experiments performed on 7500 ppm ClF_3 in ambient air (relative humidity 65%) indicated that approximately 85% of the ClF_3 had reacted within 6 seconds.

Dilutions of ClF_3 with air at 50% relative humidity were made in a Teflon® bag. Infrared absorption curves were run on samples removed from these dilutions within 30 seconds after preparation. No evidence of ClF_3 was found at 5000, 2000 and 1000 ppm nominal concentrations. The evidence is strong that ClF_3 reacts very quickly with moisture in the air to form materials with little or no IR absorption, such as HF or Cl_2.

Analysis of Hydrogen Fluoride

Since it was possible that HF was the major toxic agent in ClF_3 exposures and since monkeys were to be exposed to HF with subsequent plethysmographic lung function measurements, a method of continuous analysis of HF using the fluoride ion specific electrode was developed. The HF is absorbed in citrate-acetate buffer and delivered to the electrode by a peristaltic pump. The fluoride electrode, along with a reference electrode, is supported in a 3/8-inch Tygon® tube through which the absorber solution flows. The apparatus is designed so that the electrode tips are completely immersed during the time of analysis. With calibration of the system before and after analysis, $\pm$ 4% precision may be attained.

Analysis of Methylisobutylketone (MIBK)

In preparation for 2-week and 90-day exposures to 100 ppm of MIBK, a gas chromatographic procedure was developed using flame ionization detection. A 10-inch column of Porapak Q operated at 190 C was used for separation of MIBK. The resulting MIBK retention time was about 1.5 minutes, and analyses could easily be performed every 5 minutes using an automatic sampling valve. Standard bags were run daily and a variation in detector response of $\pm$ 5% was found. The variation from one bag to another when run the same day was about $\pm$ 2%. Peak height is directly proportional to concentration from zero to 300 ppm.

Methemoglobin Analysis

Some of the chemical compounds under investigation in this laboratory were known to produce methemoglobin in the blood of certain animal species. Therefore, it became desirable to have a dependable method for the quantitative analysis of this modified blood pigment. The method of Evelyn and Malloy (reference 9) was found satisfactory when high levels of methemoglobin were produced by the inhalation exposure of animals to the chemical being studied. When toxicity tests were conducted at low atmospheric concentrations of contaminant near the no-effect level, the spectrophotometric method of Evelyn and Malloy was unsatisfactory due to increased turbidity in the hemolysate. This turbidity was subsequently identified as Heinz bodies from red blood cells resulting from the chemical toxicity of the test agent. The Evelyn and Malloy method was modified using the suggestions of Henry (reference 21) and Hainline (reference 17) and other procedures developed in our laboratory.

Methemoglobin has spectrophotometric absorbance peaks in the visible range at wavelengths of 630 nm and 503 nm. Oxyhemoglobin and cyanmethemoglobin have very low absorbance at 630 nm and therefore do not interfere with the measurement of methemoglobin at this wavelength. The methemoglobin concentration is determined by conversion to cyanmethemoglobin with KCN and measurement of the decrease in absorbance of the solution at 630 nm. Sulfhemoglobin, which also absorbs light at the 630 nm wavelength, does not react with KCN and, therefore, does not interfere with the measurement of methemoglobin. The addition of a phosphate buffer (pH 6.6) maintains the methemoglobin absorbance peak at 630 nm.

The turbidity effect is minimized by the addition of a detergent to the buffer solution, centrifugation of the hemolysate, and correction by subtraction of the absorbance resulting from turbidity (read at 720 nm) from the readings made at 630 nm.

A standard methemoglobin solution is made by the conversion of oxyhemoglobin by reaction with $K_3Fe(CN)_6$. The absorbance of the standard is measured at 630 nm to provide a constant (K) relating absorbance to concentration. The linearity of the absorbance curve produced was verified by using dilutions of methemoglobin produced in vitro and in vivo by reaction of oxyhemoglobin with NF_3. The formula used for calculation of methemoglobin concentration is as follows:

$$\text{Methemoglobin Conc.} = (A^1_{630\ nm} - A^2_{630\ nm}) - (A^1_{720\ nm} - A^2_{720\ nm}) \times K$$

Since there is individual animal and species variability in the completeness of hemolysis, the determined value must be corrected for loss of hemoglobin on clarification of the solution. The validity of this correction is dependent on the assumption that there is no significant difference with respect to methemoglobin content between lysed and unlysed cells. This correction is calculated as follows:

$$\text{True value} = \text{determined value} \times \frac{\text{total hemoglobin}}{\text{hemolysate hemoglobin}}$$

The method was tested on rats injected i.p. with NF_3 and has been used for analysis of blood from rats, dogs, monkeys, and humans exposed to methemoglobin producing chemicals.

In Vitro Studies on the Reaction of Dog Hemoglobin with MMH

Monomethylhydrazine (MMH), an important rocket propellant, was shown to be highly toxic by Jacobson (reference 25) and Haun (reference 18). Although acute toxicity appears related to central nervous system damage, an interesting concurrent reaction of physiological importance in some species is the conversion of hemoglobin to methemoglobin in the presence of this strong reducing agent as shown by Fortney (reference 11) and by Clark (references 4 and 5).

Species difference of methemoglobin formation has been reported both in vivo and in vitro by Clark (reference 5). The identity of the product formed in blood has been questioned. Previous studies had been performed on the reaction of MMH with diluted hemoglobin, but none controlled oxygen concentrations during the reaction.

This study originally was planned to investigate reaction rates and equilibrium concentrations of reactants and products with hemoglobin at physiological concentrations and MMH.

The MMH used in this study was obtained from Olin Matheson Company and later from Matheson, Coleman and Bell. The supply was kept under nitrogen to prevent oxidation, and only colorless MMH was used. Dilutions were made in 0.2 N HCl.

Pooled blood from five or more normal stock dogs was used within a week of the date it was drawn. It was generally used as whole blood, but a limited number of experiments were carried out using washed red cells suspended in a phosphate buffer at pH 7.4 and also using hemoglobin solutions of approximately physiological concentrations. Deoxygenated blood was prepared for use in these studies by alternate vacuum flushing of air in the reaction vessel and replacement of the atmosphere with helium.

A Perkin-Elmer 350 recording spectrophotometer was used in the absorbance mode. The course of the reaction was followed by making dilutions (1/100) of the reaction mixture in M/60 phosphate buffer (pH 6.6) at specified intervals and recording the 750-450 nm portion of the visible spectrum before and after the addition of KCN. Methemoglobin was measured by the method previously described. A methemoglobin dilution of 1/1000 was used for recording the Soret band when desired.

Usually 5 ml of blood (40-50 μ moles with respect to heme) was reacted with MMH (44, 22 and 11 μ moles). The reaction mixture, about 0.5 cm in depth, was stirred constantly and the reaction was carried out at room temperature. Tests on the MMH-hemoglobin reaction in air were conducted in open beakers, whereas the tests on anaerobic reactions were performed in sealed serum vials made anaerobic by the vacuum flushing technique. Samples for spectrophotometric analysis were removed from the sealed reaction vial by means of a syringe. A number of tests were also conducted to evaluate the effect of varying oxygen concentrations on the MMH-hemoglobin reaction. These tests were performed in a manner similar to the oxygen free studies, that is, removal of air and its replacement with an oxygen-helium mixture.

As the aerobic MMH-hemoglobin reaction proceeded, the mixture turned from bright red to brown and an observable release of gas occurred. With MMH to heme ratios greater than 2:1 there was rapid denaturation and precipitation. Dilution spectra at pH 6.6 have maxima and minima similar to those of methemoglobin as shown in figure 2. The 630 nm spectrophotometric peak is pH dependent and the spectrum changes to that of cyanmethemoglobin with addition of KCN. Comparable spectra are also obtained from mixtures of hemoglobin and methemoglobin solutions. These observations support previous work (reference 4, 5 and 11) in identifying the compound as methemoglobin.

In the anaerobic reaction there is apparently little change beyond that supported by the trace of oxygen remaining after deoxygenation or that little introduced with MMH solution as shown in figure 3. The spectrum of reduced hemoglobin containing a slight amount of methemoglobin is observed with careful dilution of the reaction mixture in deoxygenated buffer. After introduction of oxygen the reaction proceeds rapidly as shown in figure 4. The MMH itself (or some nonidentified reactive intermediate compound) is apparently stable in blood for prolonged periods in the absence of available oxygen.

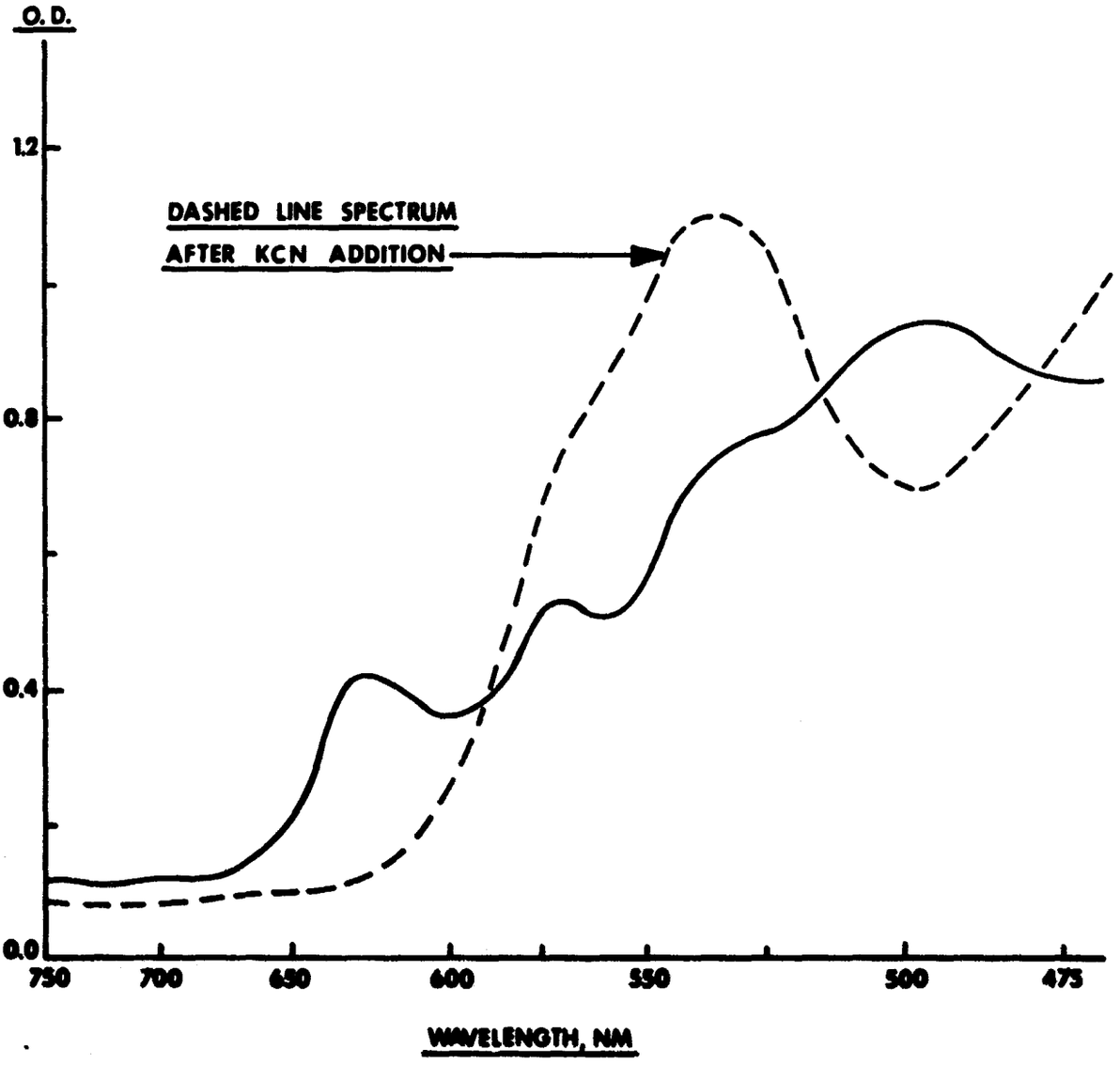


Figure 2

Conversion of Hemoglobin to Methemoglobin by MMH

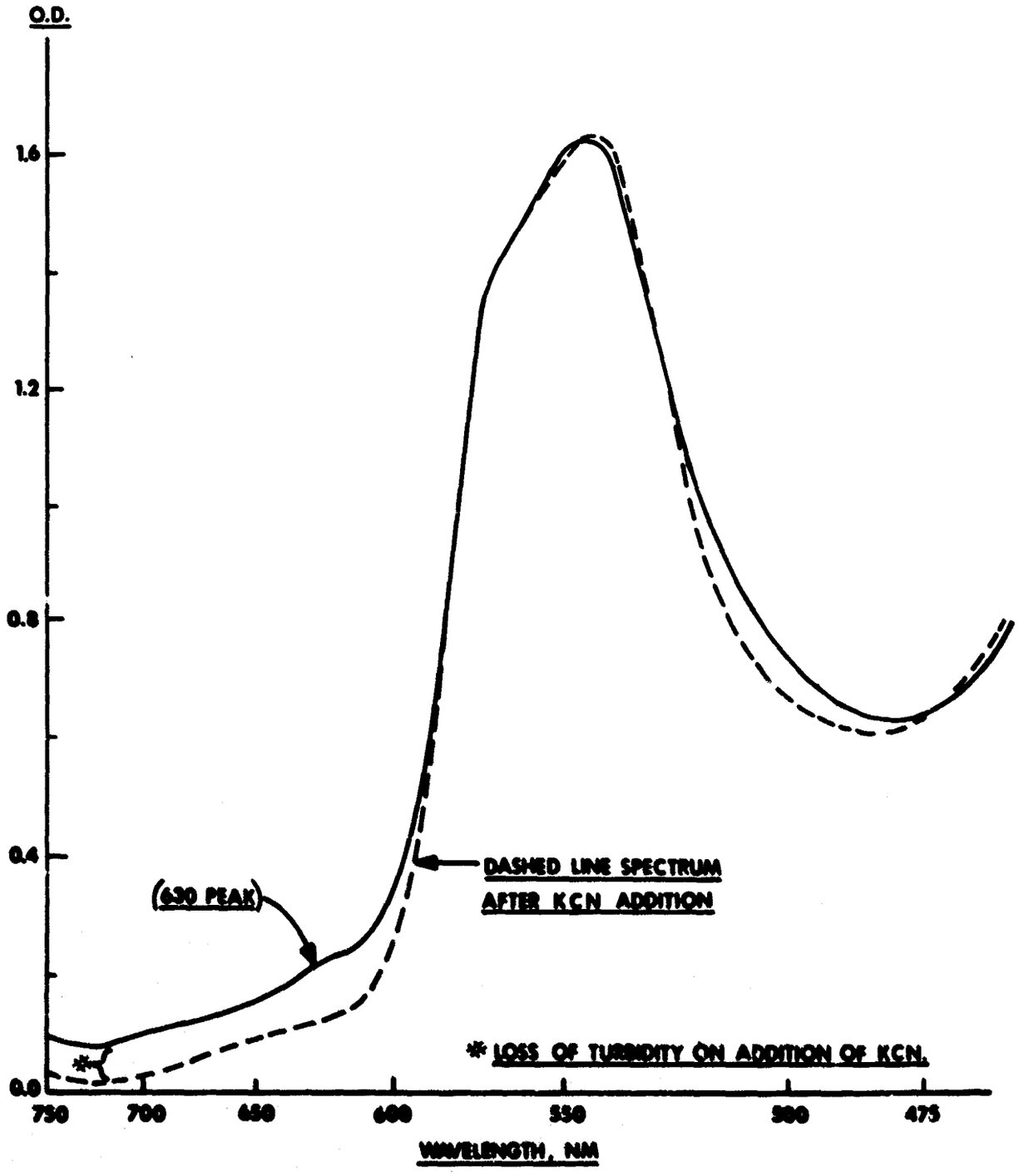
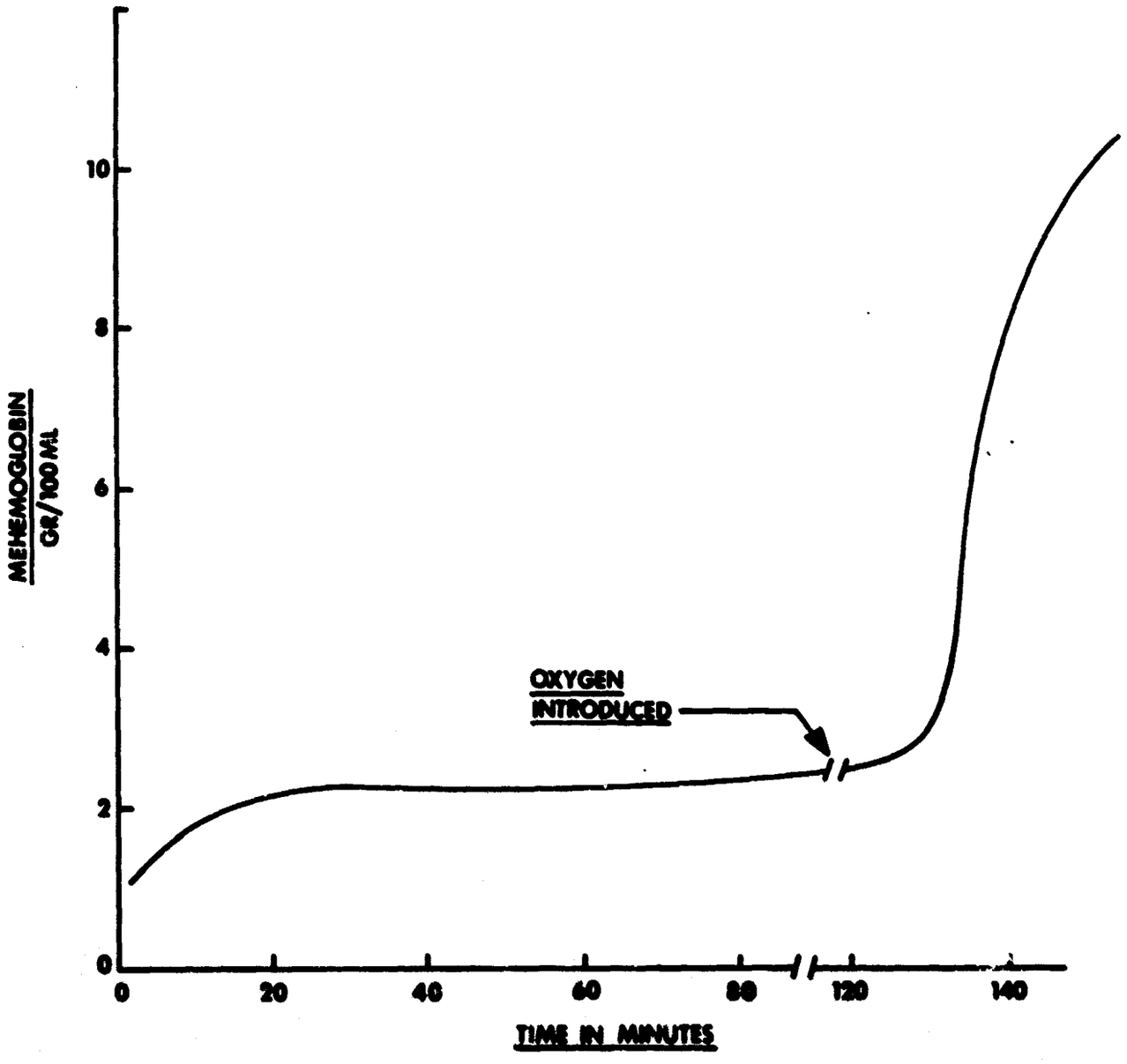


Figure 3

Effect of MMH on Reduced Hemoglobin



METHEMOGLOBIN FORMATION RATE

Figure 4
Methemoglobin Formation Rate

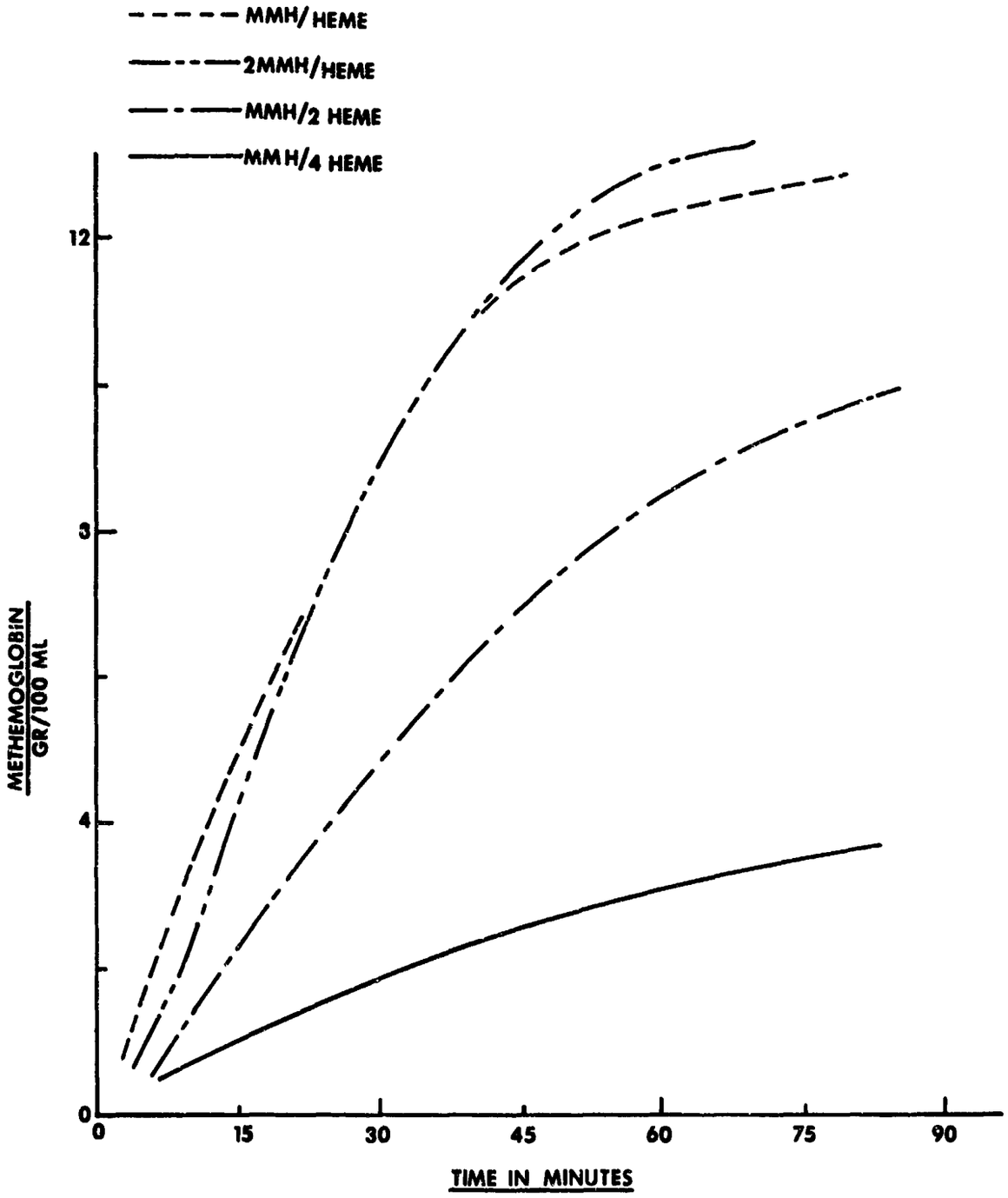
MMH reaction rates measured by methemoglobin formation (hemoglobin held constant and unlimited air available) appear dependent directly on MMH concentration up to an equimolar MMH to heme ratio as shown in figure 5. The limit appears to be approximately 80% conversion of hemoglobin to methemoglobin, at which point the protein denaturation becomes significant and one can no longer measure total hemoglobin using the cyanmethemoglobin method. With lower MMH concentrations the equilibrium coversion ratio appears to approach 2 moles of heme oxidized per mole MMH consumed.

Quantitation of the involvement of oxygen in the reaction was attempted using gas chromatography. A 4-foot, 5 A molecular sieve column at 62 C was used with a helium flow rate of 77 cc/minute and a nickel hot wire thermal conductivity detector operating at 250 milliamps. Qualitatively, oxygen is consumed and nitrogen and methane are produced. Calculations on limited data suggest a breakdown of MMH similar to that reported by Vernot et al. (reference 49). The molar ratio of N_2 to CH_4 is 5 to 1 and N_2 to O_2 approximately 1 to 1.

Analysis of Thomas Dome Atmospheres

In toxicological exposures of large numbers of animals in the Thomas Domes, concern has been expressed about the possibility of toxic effects resulting from the build-up of contaminants from the animals themselves. This concern has resulted in extensive discussion on the possible existence of unknown toxic materials in the dome atmosphere (reference 35 and 50). Most investigators concerned with the determination of contaminants in confined spaces have dealt with human subjects (reference 36) and gas-off products from construction materials (reference 23). The list of products or contaminants found in habitated confined spaces is quite formidable (reference 22). The confinement of animals, however, has the additional aspect of biological waste as a contaminating factor. While there has been considerable work done on confined atmospheres, biological waste products have usually not been important, and atmospheric pollution from animal waste has received little attention.

The enclosure of animals in an inhalation exposure chamber for toxicity investigations results in the production of a characteristic odor which is not easily eliminated without the use of prohibitively high air exchange rates. The odors are most readily noticed in exposure chambers such as the Thomas Domes where research personnel enter the chamber to make biological measurements on the animals and to provide routine animal care. These odors appear to be metabolic waste products of the animals, possibly combined with oxidation products of body wastes. Subjectively, the odors are most noticeable when the domes are operated at reduced pressure conditions (68% O_2 - 32% N_2 at 5 psia pressure).



EFFECT OF VARIATION IN MMH CONCENTRATION ON METHEMOGLOBIN FORMATION RATE

Figure 5

Effect of Variation in MMH Concentration on Methemoglobin Formation Rate

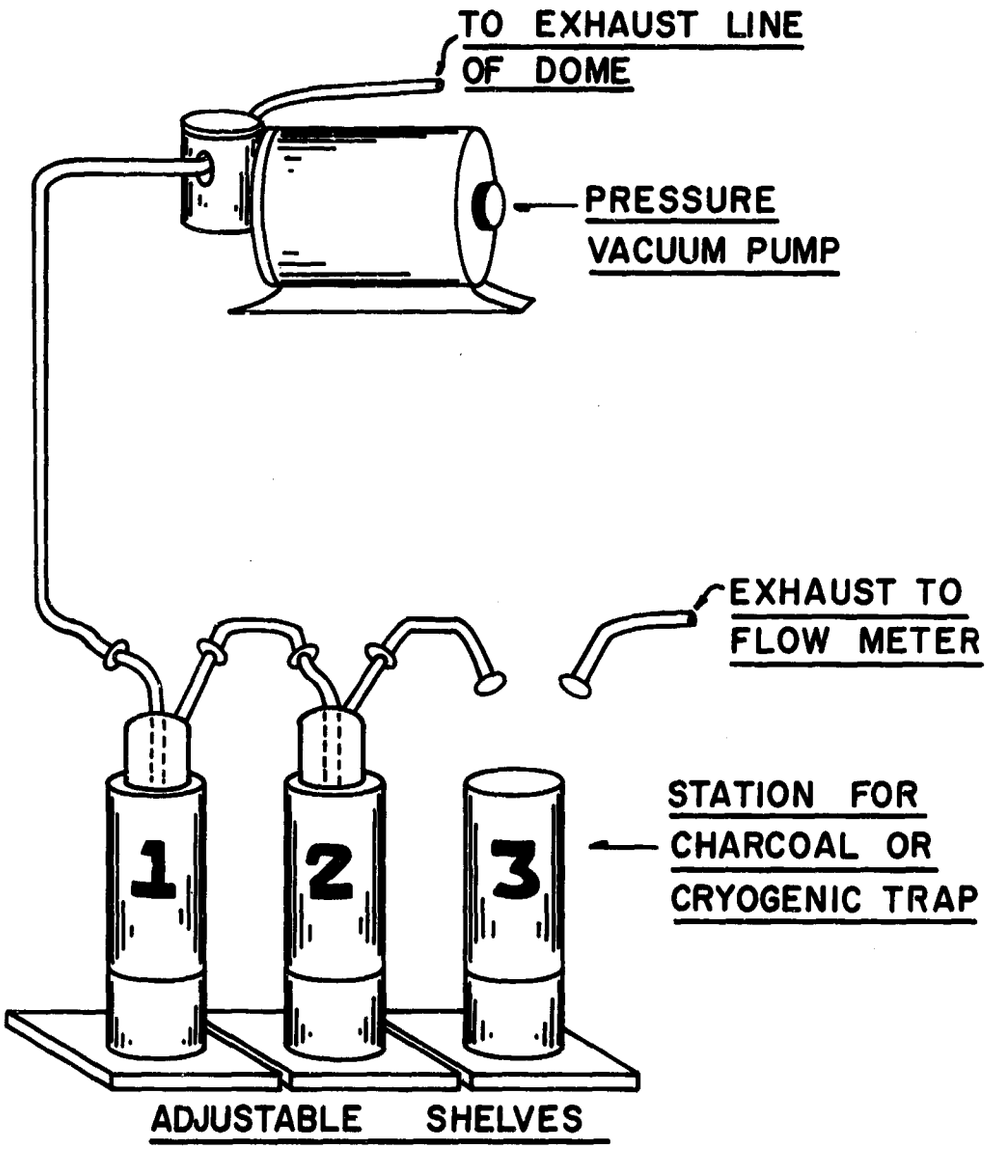
To provide a clean environment for the animals, the dome atmospheres are replaced at a flow rate of 120 cfm (of rarified air) or sufficient for a complete changeover every 7 minutes. The domes which have an approximate volume of 875 cubic feet are thoroughly cleaned once a day and the floor flushed with water once or twice during each 8-hour shift period.

A series of experiments was initiated to define the composition of the dome odor. The initial efforts were directed toward finding a satisfactory method for collection and concentration of the unknown dome contaminants for subsequent analysis. Gas chromatography was the most sensitive technique available to determine whether contaminants existed in a dome operated under these conditions. Other techniques were tried but essentially without results. The presence of some alkaline material was detected by passing the dome atmosphere through a standard acid solution and ammonia was identified by colorimetry. Otherwise, gas chromatography was used in this study. The collection of samples required a pump to withdraw atmosphere from the partial vacuum in the dome and pressurize it to ambient. When this was done, the sample became supersaturated with moisture so that liquid as well as gaseous samples were collected.

A gas chromatograph equipped with a nickel hot wire thermal conductivity detector was used in a series of preliminary studies. The nickel hot wire was less adversely affected by large concentrations of air than the usual tungsten detectors and was capable of detecting materials at concentrations of as little as 100 ppm of any atmosphere. In the dome studies from which air samples were collected sufficient carbon monoxide was added to the atmosphere to provide a dome concentration of 440 mg/M^3 which was easily measurable by direct injection into the chromatograph.

In addition to the chromatograph equipped with the thermal conductivity detector, other instruments equipped with flame ionization detectors were used. The flame ionization detector is much more sensitive to organic materials but is insensitive to any inorganic gas including carbon monoxide. Auerbach and Russel (reference 1) have shown that methane was generated at a greater rate than most other gases in human confinement studies. Methane is also present at 5-15 ppm in the oxygen that is used in the dome atmosphere. Preliminary studies of the dome atmosphere involved direct injection into the gas chromatograph with the flame ionization detector. Only methane was present in sufficient quantity to be detected by this technique.

Trapping techniques were used to concentrate the dome impurities, but the presence of large amounts of moisture prevented this from being a straightforward procedure. Charcoal adsorption and direct cryogenic trapping with liquid nitrogen were the techniques finally selected for use. Figure 6 shows a trapping system that permitted the use of one or two pretraps to remove most of the water from samples taken from the dome during toxicity studies.



ATMOSPHERE SAMPLING TRAP SYSTEM

Figure 6

Atmosphere Sampling Trap System

Preliminary direct injections of the dome atmosphere into the gas chromatographs served to indicate the level of contaminant concentrations to be expected. Since most organic substances could have been detected using the flame detector at a level of about 1 ppm or greater and none were seen, it is concluded that no organic materials other than methane were present in the dome atmosphere at a concentration above 1 ppm. In spite of the low level of materials present, there was a disagreeable odor in the dome. W. E. Burnett (reference 3) pointed out that some materials of animal origin are capable of contributing odor at extremely low concentrations. Burnett used an effluent splitter with 20% of the effluent going into a flame ionization detector; the other 80% was sniffed by a laboratory worker who indicated odors as they came off the column. Figure 7 illustrates the gas chromatographic peaks and odors detected by Burnett. Some materials produced odor but no peak. Since one of the principal odorous constitutents of feces, skatole, is reported to have an odor threshold of 0.000075 ppb, this is not surprising.

A sample concentrating device was required to obtain additional information. To accomplish this a charcoal trap was devised that consisted of a 3/4" OD stainless steel tube 12" long. This tube was packed with 30 g of coconut charcoal and equipped with fittings to allow installation in the trapping system or in a tube furnace for conditioning or desorbing. Trapping was usually done overnight in a system that utilized one or two pretraps for water removal. The samples were desorbed from the charcoal by vacuum distillation and collected in an ice water trap followed by a liquid nitrogen trap. Essentially the same chromatograms were obtained from both traps as shown in figures 8 and 9 but a larger quantity of sample was collected in the liquid N_2 trap. Several of the peaks have been attributed to low molecular weight hydrocarbons. The peaks attributed to acetone and methylene chloride were artifacts, however, having been introduced when some of the equipment was cleaned with these solvents. The charcoal trap preferentially absorbed low molecular weight nonpolar materials and, therefore, could not be depended upon alone for quantitative estimation of all the contaminants.

Temperature programmed gas chromatography of both the aqueous and head space phases in the traps was conducted and chromatograms similar to figures 10 and 11 obtained. This technique revealed more contaminant than the isothermal gas chromatograms. As shown in figure 11, 30 peaks were obtained. Not all the peaks produced were from materials generated in the dome, however. Various materials were introduced from the normal outdoor air mixed with oxygen to produce the 68% O_2 - 32% N_2 dome atmosphere. The peaks in figure 11 that are numbered 11, 21, 25 and 29 matched those found in outdoor air with respect to retention times and peak heights. Peak number 9 and a small portion of peak 23 were

Figure 7

Organoleptic Evaluation of Gas Chromatographic
Fractions of Liquid Manure Volatiles

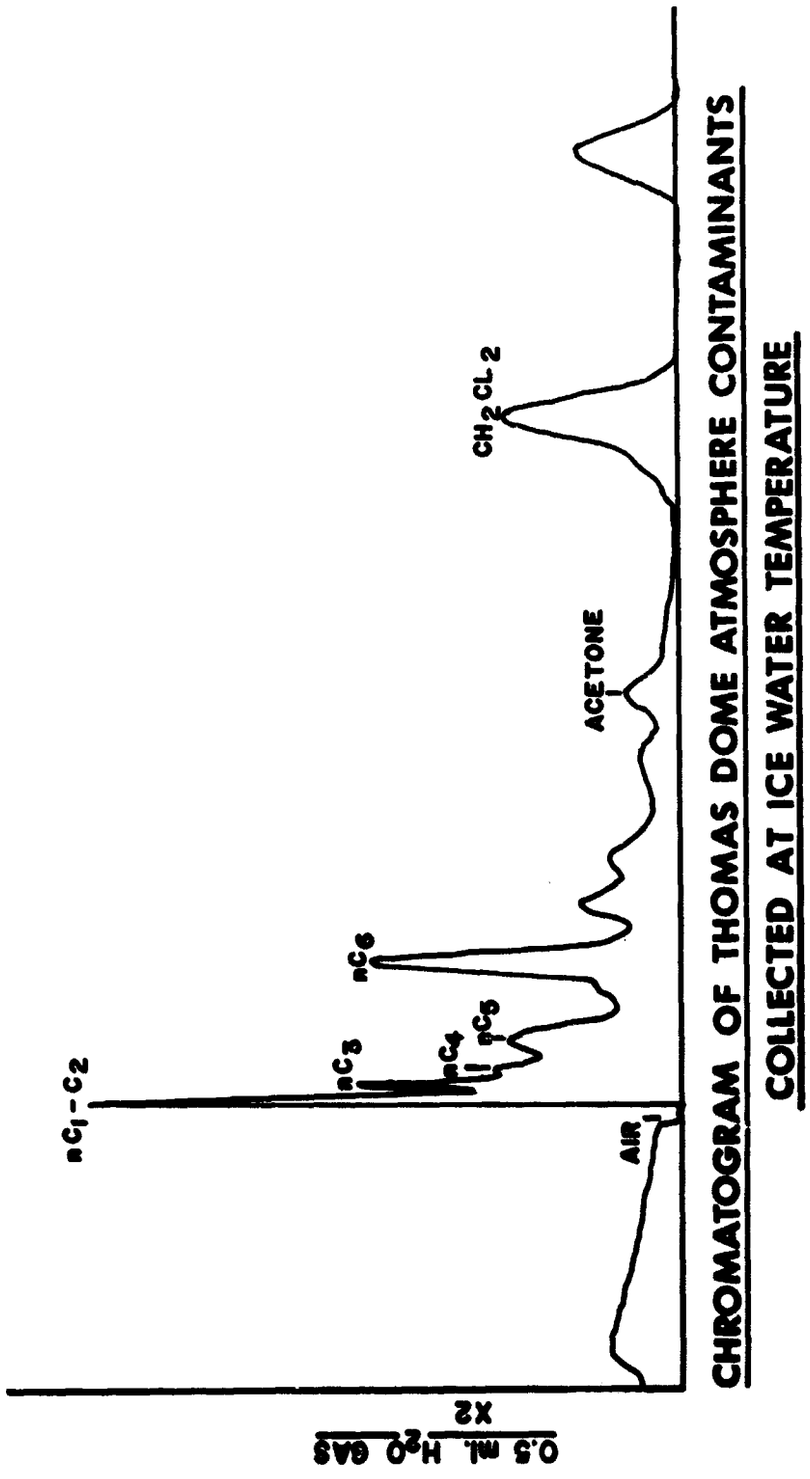


Figure 8

Chromatogram of Thomas Dome Atmosphere Contaminants
Collected at Ice Water Temperature

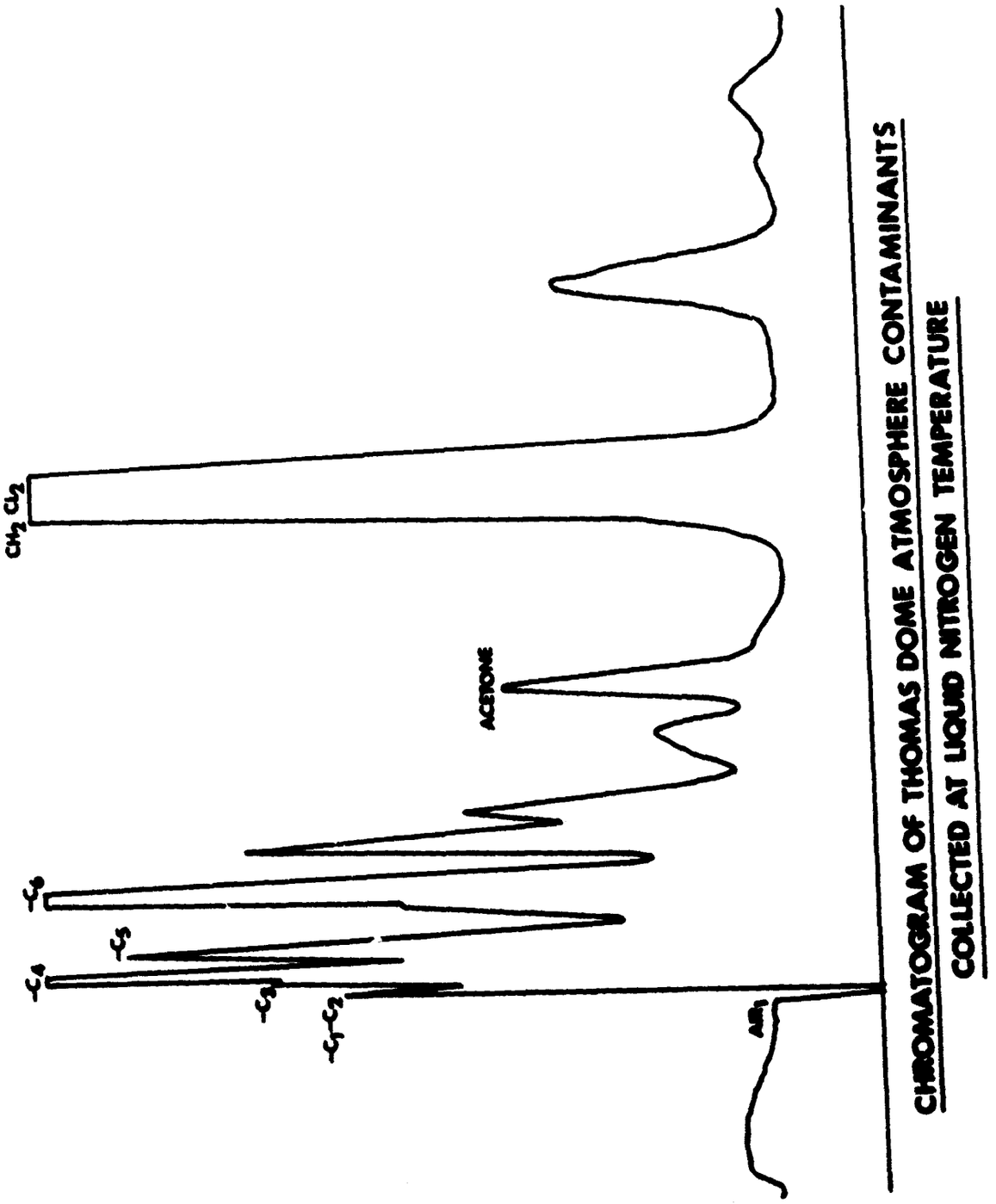


Figure 9

Chromatogram of Thomas Dome Atmosphere Contaminants
Collected at Liquid Nitrogen Temperature

Figure 10

Chromatogram of Thomas Dome Atmosphere Contaminants
Collected in Water Condensate Sample

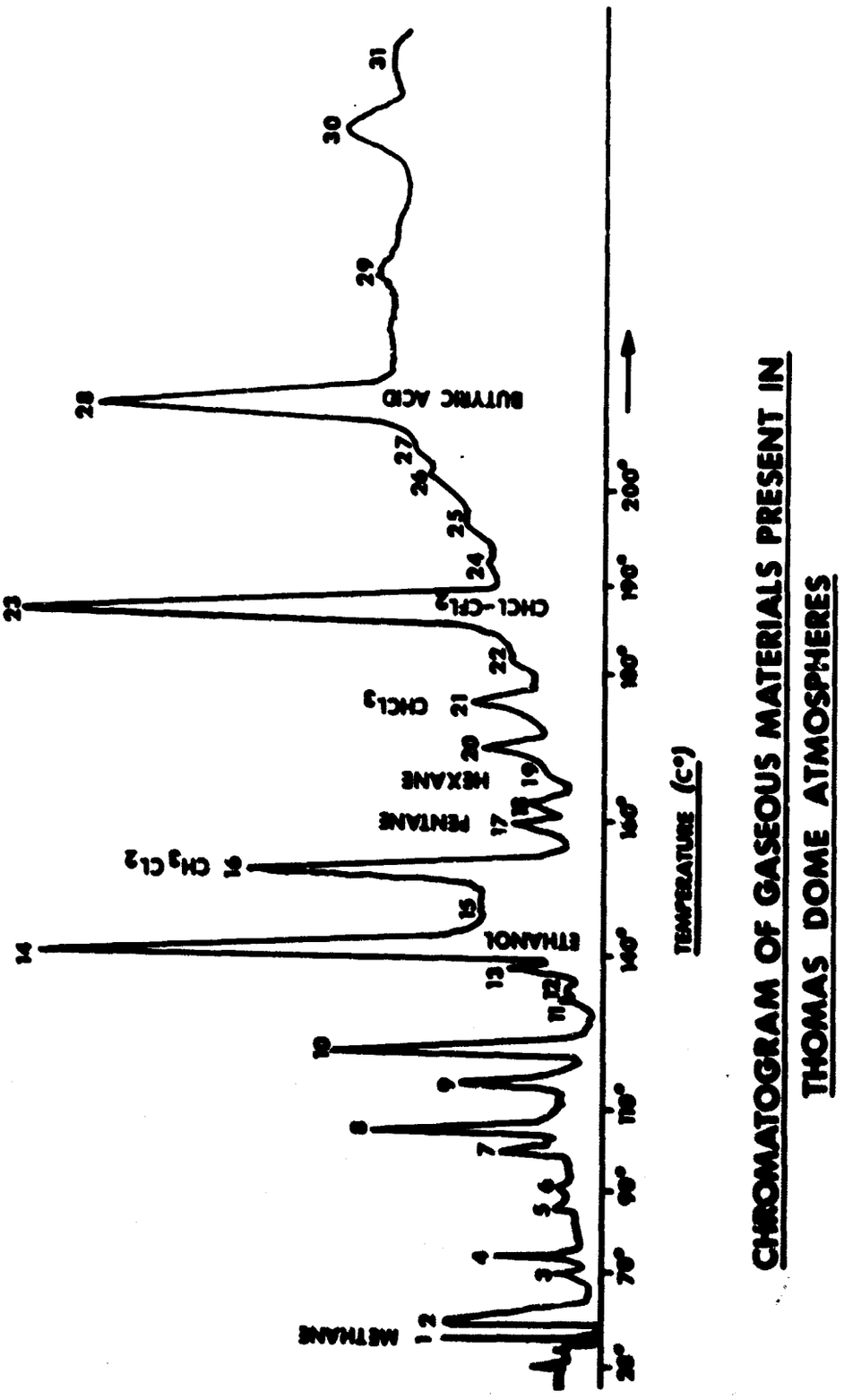


Figure 11

Chromatogram of Gaseous Materials Present in Thomas Dome Atmospheres

introduced by contamination from the chromatograph septum. All peak identifications were made by matching retention times at several temperatures with those of known compounds. In repetitive samples the peak intensities varied from day to day but the total concentration remained very low. The total concentration of organic contaminants (aside from methane) is less than two parts per million of the dome atmosphere.

The threshold limit values (TLV) of some of the materials that have been identified in human confinement studies compared with those found in the Thomas Domes are shown in table I. Retention identification indicates that the dome contaminants are not highly toxic materials and are present in such extremely low concentrations that they should have no physiological effects on the experimental animals housed in these chambers.

ENGINEERING PROGRAMS

The major emphasis of the Facility Engineering Department during the past report period has been in the organization of responsibilities to obtain efficient accomplishment of assigned projects. The main areas of responsibility are Preventive Maintenance, Corrective Maintenance and Project Construction. These categories have been scheduled to provide complete preventive maintenance coverage and also accomplish project support of the facility.

The primary concern in accomplishing these objectives was to attain maximum effectiveness of THRU personnel with a minimum of clerical and supervisory requirements. Special procedures were developed.

All items of equipment in the facility were scheduled for periodic preventive maintenance. These items are subdivided into systems and assigned to specific technicians for service. The lists of equipment are posted in the work areas and the assigned individuals are responsible for their respective lists. Corrective maintenance required for this equipment is processed by Air Force Form 211 through the appropriate supervisor. Equipment calibration is considered a function of preventive maintenance and is also scheduled on these charts.

A separate format was devised to cover tasks at a project level. A form designated as the Facility Engineering Work Request was devised to provide administrative control of all Facility Engineering tasks not related to preventive maintenance. This form is completed and forwarded to the Facility Engineering Department for approvals and accomplishment of the request.

TABLE I

Comparison of Thomas Dome Atmosphere Contaminants
with Threshold Limit Values

	TLV	Amount Found
Methane	asphyxiant	17-19 ppm
Ammonia	50 ppm	0.5 ppm[1]
Chloroform	50 ppm	35 ppb
Methylene Chloride	500 ppm	40 ppb
Trichloroethylene	100 ppm	10 ppb[2]
n-Butyric Acid	10 ppm	15 ppb
Ethyl Alcohol	1000 ppm	0.25 ppm
m-Xylene	100 ppm	10 ppb[2]
Ethyl Mercaptan	10 ppm	nd[3]
Methyl Amine	10 ppm	nd[3]
2-Butanone	200 ppm	nd[3]
n-Propylacetate	200 ppm	nd[3]
Ethyl Ether	400 ppm	nd[3]
Acetaldehyde	200 ppm	nd[3]

By utilizing the total peak area and the relative sensitivity of methane, the total organic vapor content of the dome atmosphere is estimated to be less than two parts per million.

[1] Estimated from alkalinity measurements.
[2] Peak area relative to methane.
[3] Not detected.

Projects during the past report period have been divided between completing auxiliary systems required of Thomas Dome Facility B and updating systems installed during the first years of operation of the Toxic Hazards Laboratory.

Oxygen Breathing System

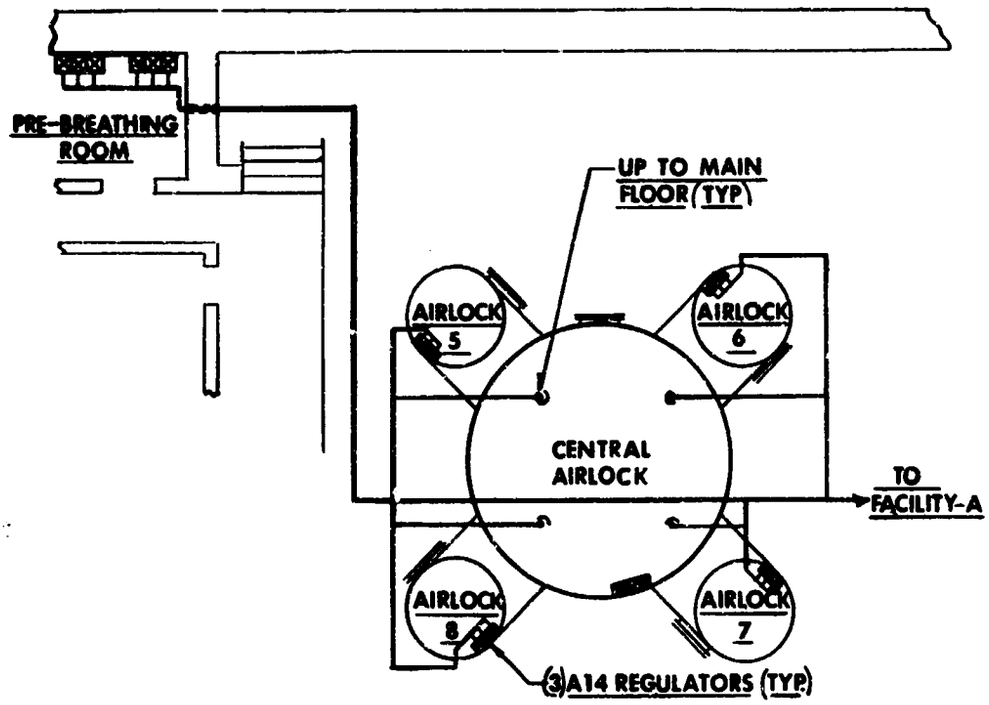
Standard Air Force A-14 O_2 breathing regulators were installed in Domes 5, 6, 7, 8 and interconnecting airlock. Each station consists of three regulators in parallel providing facilities for three individuals. In addition six regulators of the same type were installed in the prebreathing room of the new facility. This provides O_2 prebreathing facilities as shown in figure 12 for at least three dome entrants for each facility simultaneously. The system is connected to the prebreathing system of Facility A providing a secondary O_2 supply in case of emergency.

Dome Communication System (Facility B)

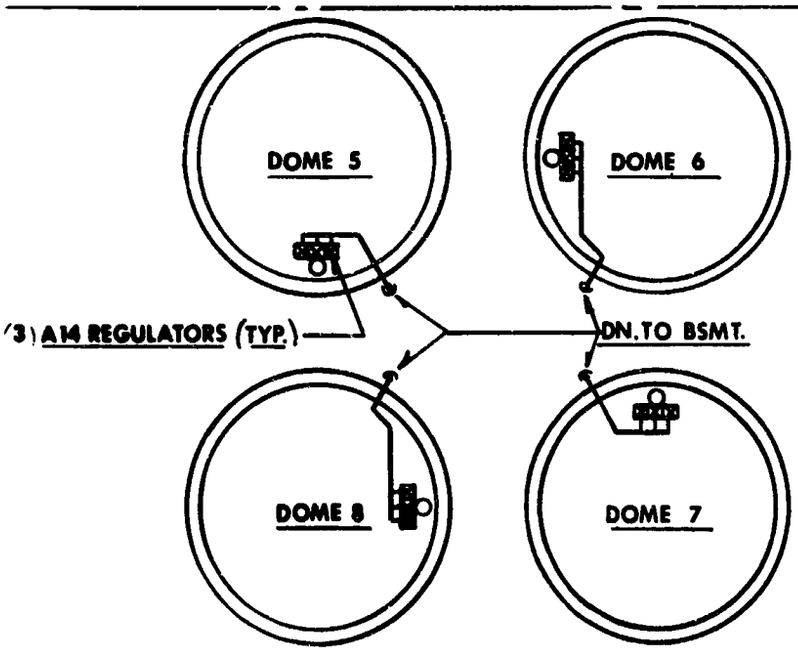
As mentioned in the previous annual report, additional equipment was installed in the dome communications system consisting of two stations around the periphery of each dome and the interconnecting airlock. An additional control station at the main control panel was also provided. This installation completed the dome communications system for Facility B as designed.

Vacuum Pump Replacement

Three vacuum pumps of increased flow capacity were procured and installed in Facility B. These pumps were Gardner-Denver, Model 5CDL-13, single stage, oil free, 100 percent oxygen compatible rated at 200 scfm. They are identical to the pumps previously installed in Facility A. Because their flow capacity was greater than the pumps replaced, all supply and discharge piping had to be redesigned. Conveniently, the new pumps were physically similar to the pumps replaced and able to be installed in the same locations as shown in figures 13 and 14. The facilities of our shop were not sufficient to fabricate the required piping so the system was designed and prefabricated components procured from outside sources. Installation was accomplished without major difficulties. The original pumps could only be controlled from an air conditioning panel in the new dome room. When these pumps were replaced, control switches were added in the basement vacuum pump room adjacent to the pumps, for use in preventive maintenance. With the addition of these pumps, the facility has a total of six pumps of the same construction providing for adequate emergency backup with allowance for equipment overhaul.



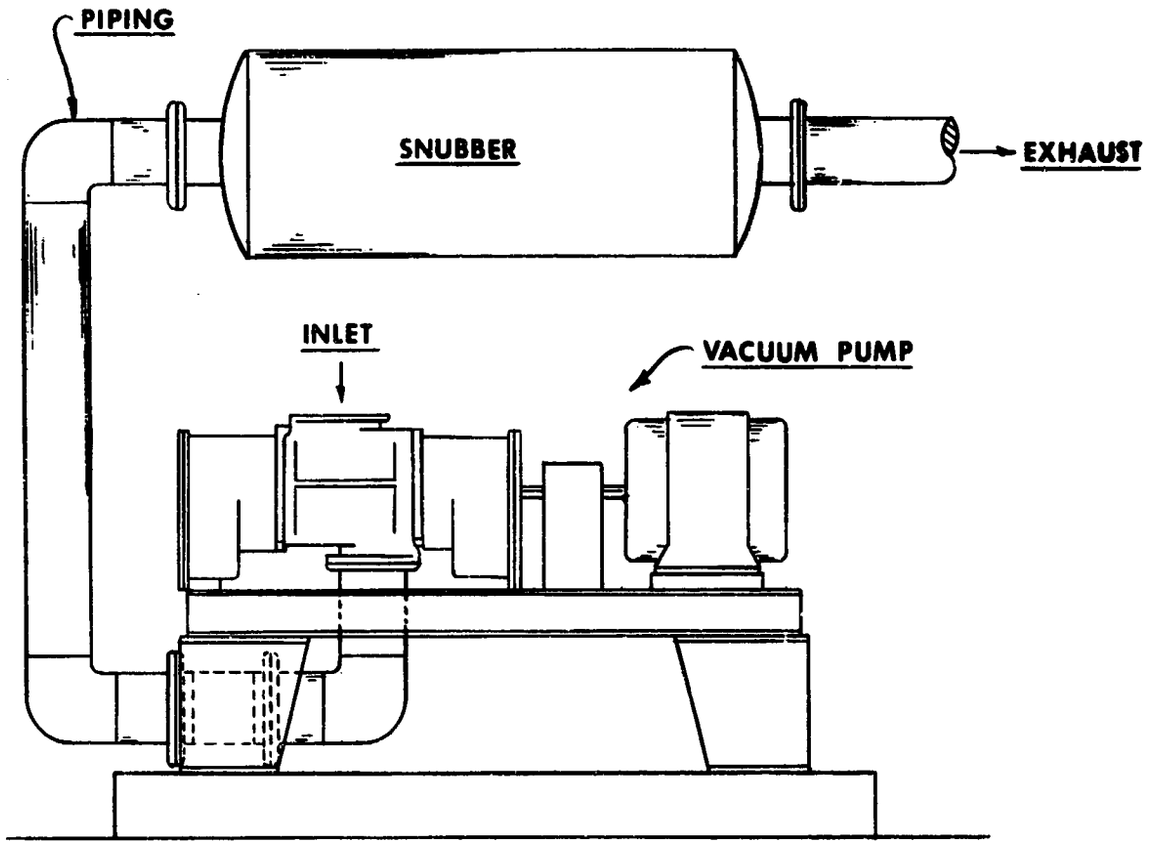
O_2 BREATHING SYSTEM-- BASEMENT



O_2 BREATHING SYSTEM--MAIN FLOOR

Figure 12

Oxygen Breathing Systems



VACUUM PUMP LAYOUT

Figure 13

Vacuum Pump Layout

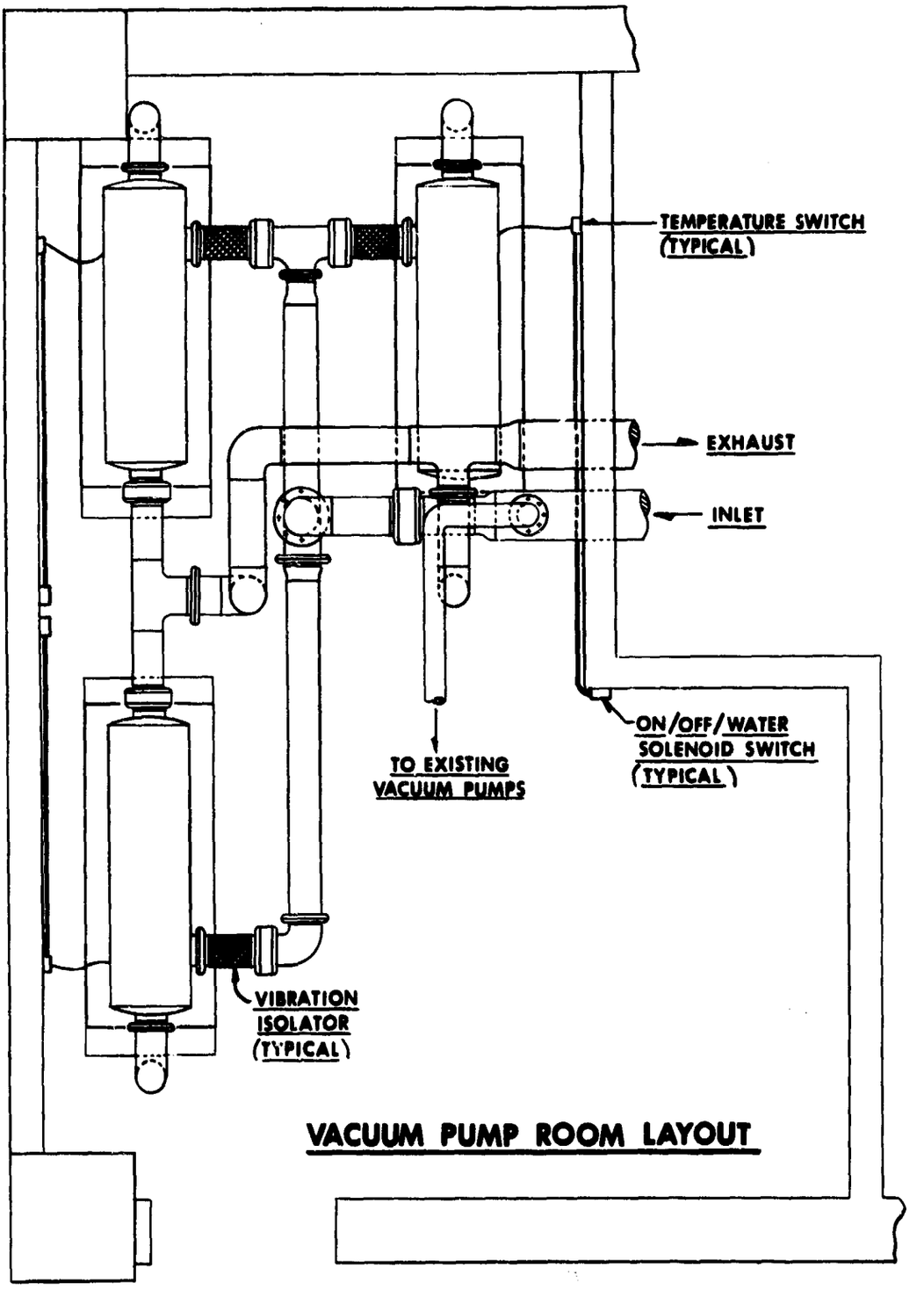


Figure 14

Vacuum Pump Room Layout

Dome Drain Valves

The drain valves installed on the domes were of the chain-operated lever type. Due to the location of these valves their operation was extremely difficult and leakage back into the domes was a continuing problem. The chain-operated levers were replaced with pneumatic operators, shown in figure 15, controlled from a convenient point adjacent to the Observer B control station at each airlock. Operation of the dome drain valves is now convenient and sure.

Contaminant Vent System

The contaminant vent system installed during the Military Construction Program was modified. The original system included a centrifugal pump to provide a negative pressure in the vent lines. Originally a closed system, an opening was provided to dilute the contaminant effluent with room air as shown in figure 16. A solenoid valve was installed at this opening to isolate the contaminant vent lines in case of pump failure resulting in loss of the line negative pressure.

Pass-Thru Airlocks

Pass-thru airlocks fabricated by the base shop were installed in each dome. Suitable controls for flushing, repressurization, and depressurization were installed. These airlocks had doors of a different design than those in the original domes. These doors leaked so badly that the pass-thru airlocks could not be used at altitude. A temporary solution has been achieved by replacing the pin-sealing mechanism with lugs tightened separately.

Emergency Solenoids - Contaminant Introduction Systems

Solenoids, similar in construction to valves on Facility A, were installed as shown in figure 17, in the toxic contaminant feed lines to each dome of Facility B. These solenoids are operated by a feedback signal from the dome flow system to route contaminant flow to vent lines in case of an emergency. This will prevent the possibility of contaminant buildup in any chamber if dome flow is interrupted.

Primate Cages

Primate cages of a design similar to the Holloman cages in Dome 3 were constructed for the domes. These cages, shown in figures 18 and 19, were designed and fabricated in the THRU shop. Cages were provided for Domes 2 and 4 in Facility A and Domes 5, 6, 7, 8 and interconnecting

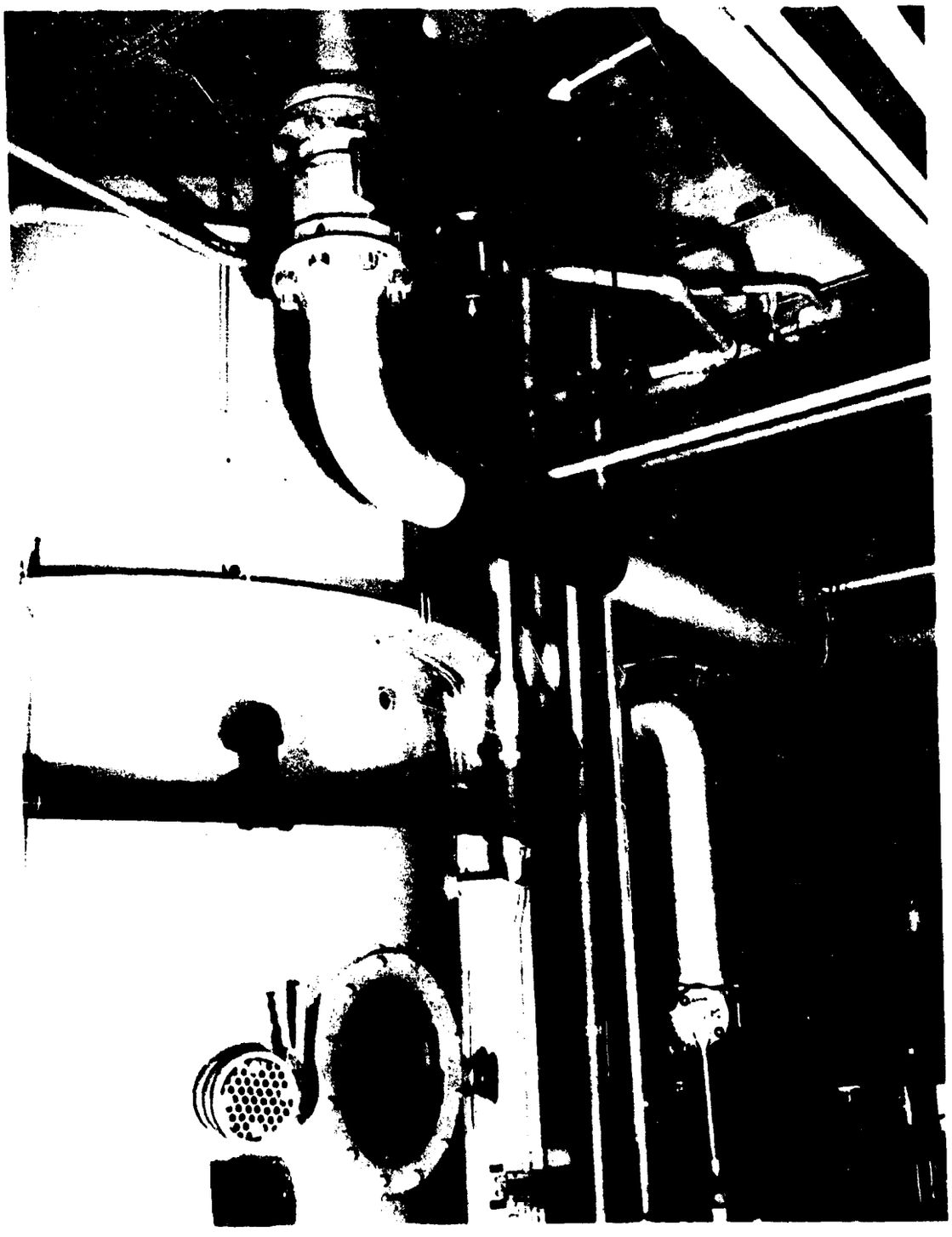
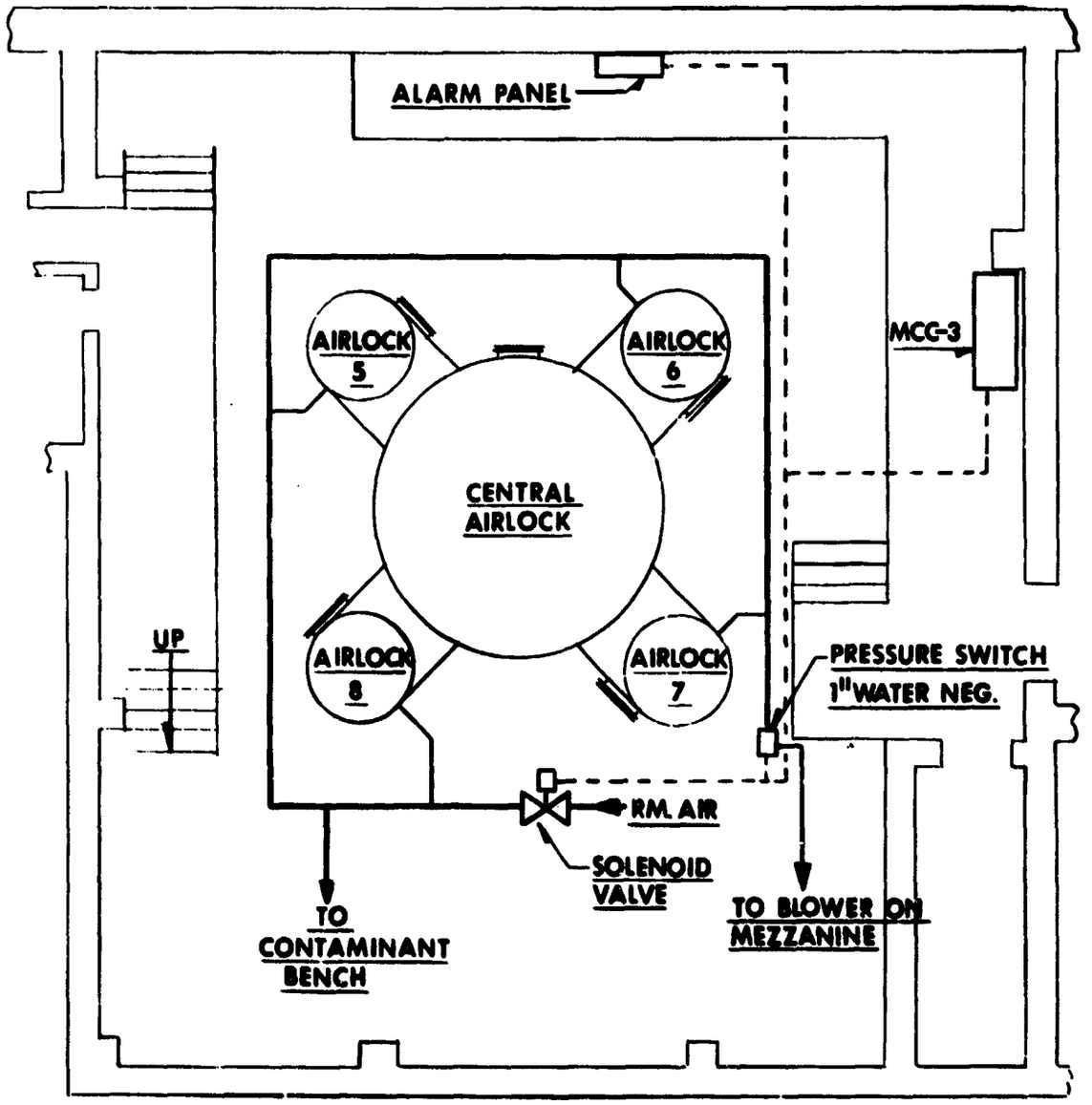


Figure 15

Dome Drain Valve Layout



CONTAMINANT VENT SYSTEM

Figure 16

Contaminant Vent System

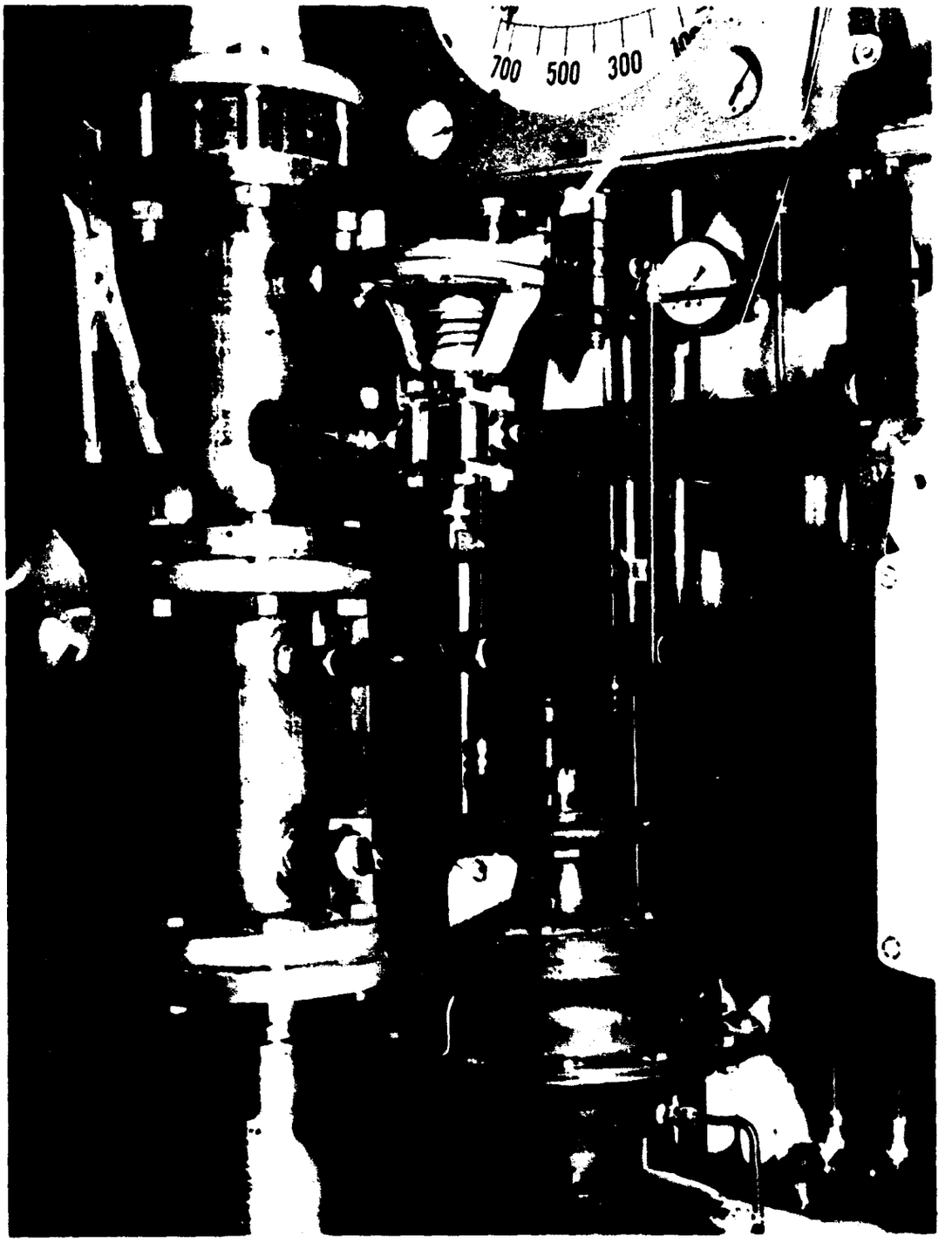


Figure 17

Emergency Solenoids--Contaminant Introduction System

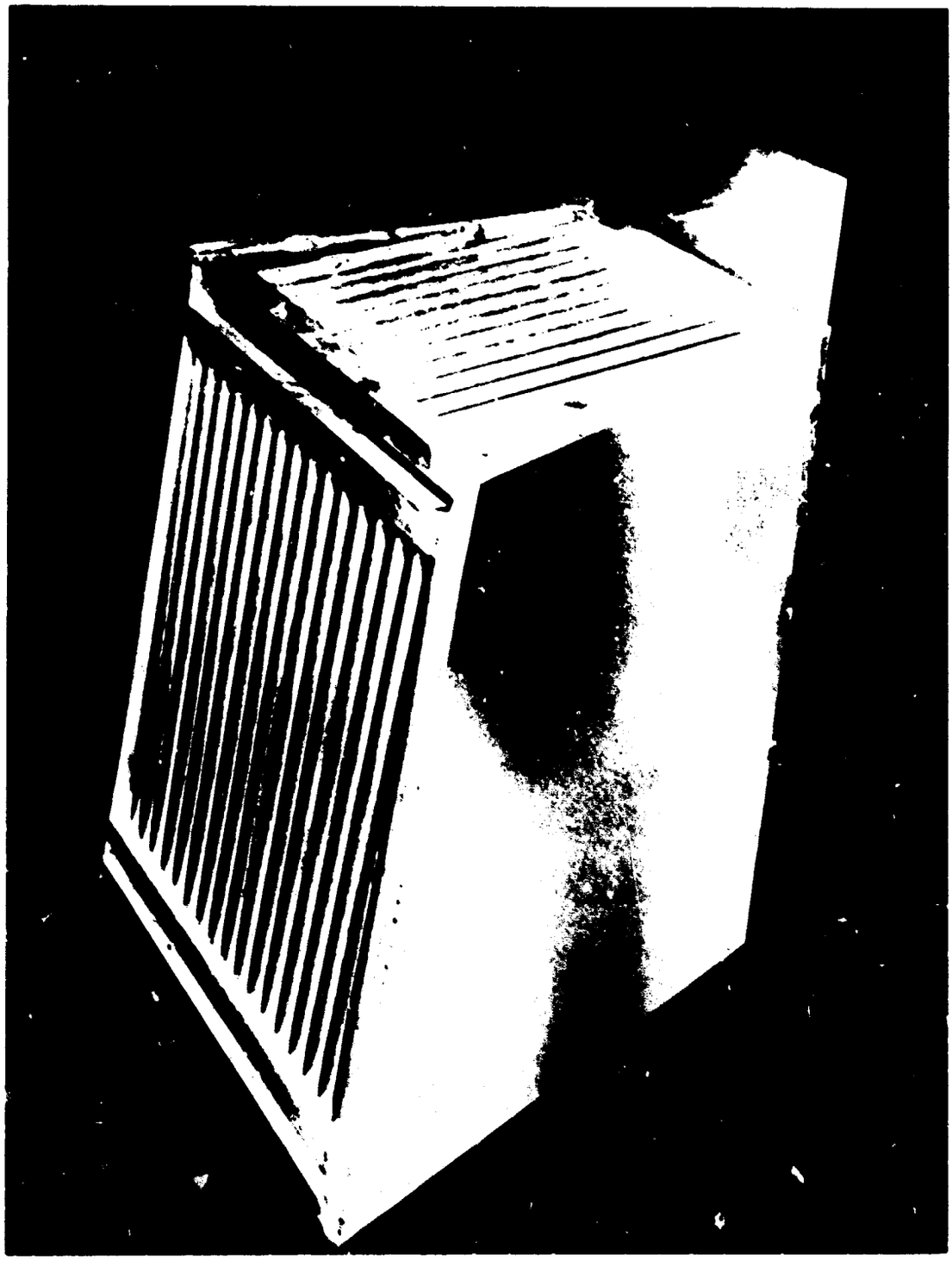


Figure 18

Dome Primate Cage - Front View

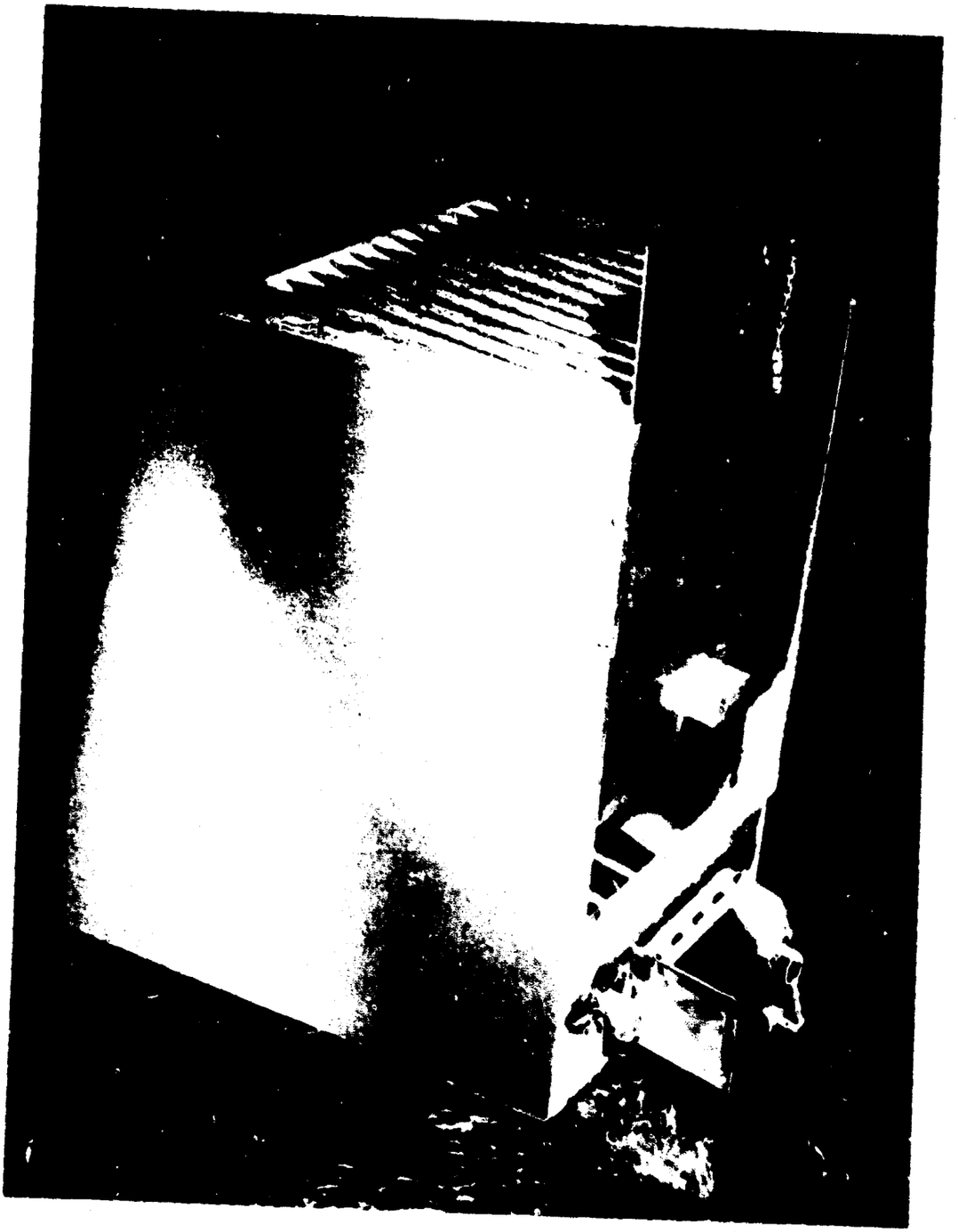


Figure 19

Dome Primate Cage - Rear View

airlock in Facility B. The original monkey cages were located on free-standing supports in each chamber. The new cages are located on racks supported from the dome wall. Practical advantages are an increase in usable floor area and easier cleaning. The cages were designed and built of heavier materials than the type previously used and should result in reduced maintenance requirements. Each unit is equipped with a standard feeder and a removable automatic watering device.

Communications System (Facility A)

A communication system was designed and installed in Facility A of the Thomas Dome altitude chambers. The advantages of the redesign system are simplified operating controls, utilization of standard Air Force communications components in the basic system, independent communications available in each dome, and automatic voice-operated tape recording of all activities utilizing the system.

The system consists basically of a station for the control panel operator, four identical dome stations, and an interface line to connect the prebreathing room station and animal weighing communication station to the operational system of Facility A. An additional station was installed at the opposite end of the control panel to provide the dome operators with independent communication capability for two simultaneous dome flights.

The station installed at the main control panel contains the power supply, tape recorder, power controls, and an automatic switch for emergency battery operation during an electrical failure. Plug-in connections are available for two headsets at this location. Switching facilities are included which enable the control panel operator to independently connect his station with any individual dome in Facility A. The tape recorders used at both control panel stations are completely voice actuated and have an automatic-reversing attachment. The tape reel used allows 6 hours of recording time which is sufficient for the longest dome flight now anticipated. A window was cut in the side of each recorder so that the dome operators could determine operational status.

Each of the four individual dome master communication stations is identical. These stations, installed on the exterior of the airlocks, are completely enclosed and have no operating controls. There are six substations containing plug-in connections for three headsets connected to each dome station, located as follows:

1. Dome Exterior; Three Substations, First Floor
2. Observer B Station; One Substation, Basement
3. Dome Interior; One Substation, First Floor
4. Dome Airlock Interior; One Substation, Basement

A separate interface line was installed to provide versatile and flexible communications to Facility A during prebreathing and animal weighing operations. These auxiliary stations, complete with plug-in connections and switching controls, also provide independent communication capability to any dome in Facility A.

Design features of the system provide technologically advanced communications to all areas of the system and complete audio capacity for all activities involving the Thomas Domes. Figure 20 shows the physical layout of components on the first floor and figure 21 shows all components in the basement areas.

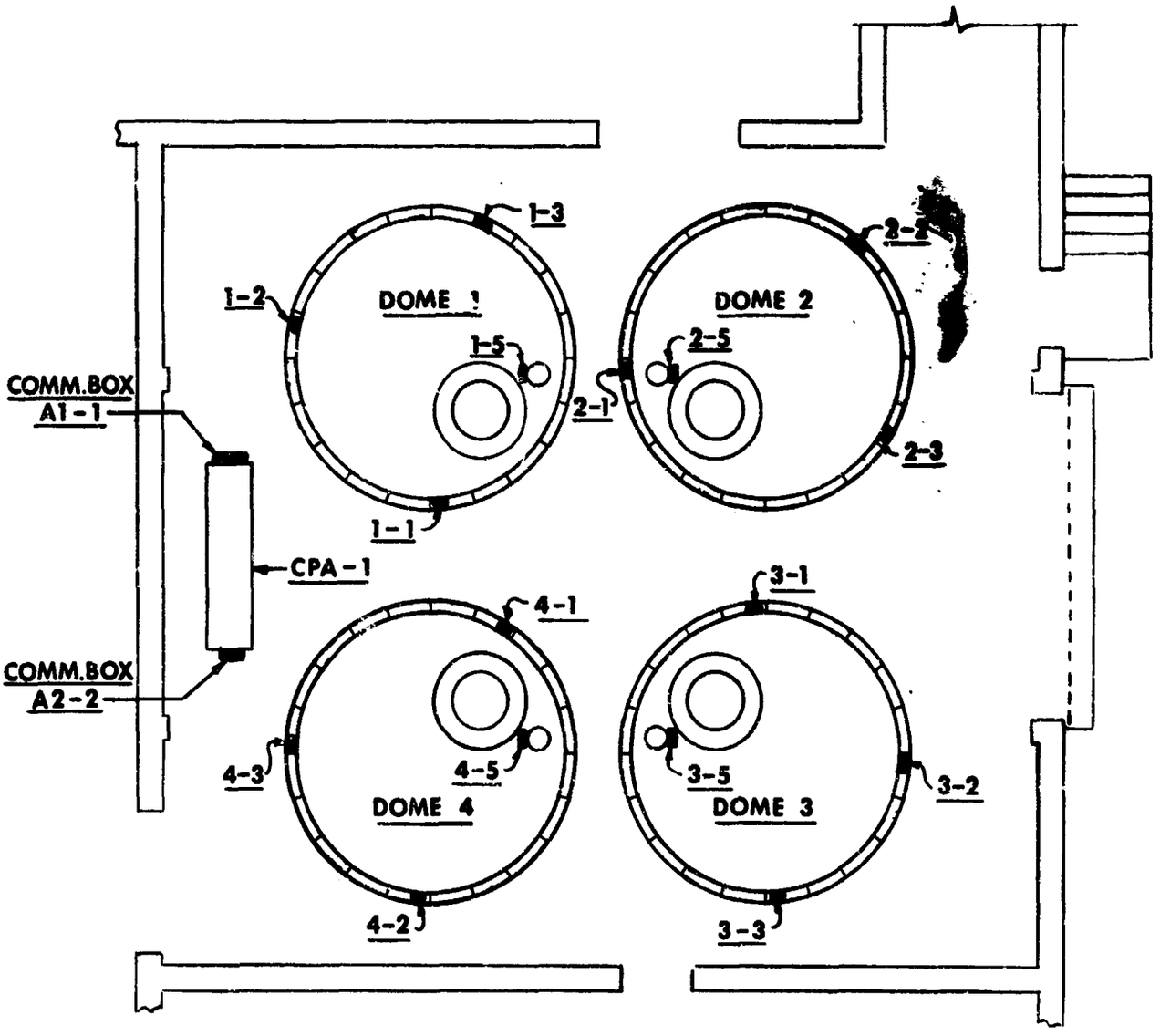
Thomas Dome Animal Weighing System

A new animal weighing system was designed and installed in both Facility A and Facility B of the Thomas Domes. Particular advantages of the new system are simplified operating controls, utilization of standarized animal holding devices, centrally located control panel, nixie tube readout, high accuracy; and an audio station capable of being independently connected to any one of eight domes or the interconnecting airlock.

The system consists basically of a control panel and 18 load cells shown in figures 22 and 23. Each dome has two weighing devices, one for weighing rodents and the other for weighing large stock animals (see figure 22). The rodents are weighed in a perforated porcelain pan which permits animal waste to fall through during the weighing operation. The monkeys are similarly weighed in a perforated stainless steel holding cage. The dogs are weighed in a specially constructed sling which supports the animal at his "underbelly" and thereby holds the dog motionless during the weighing operation.

Due to space limitations inside of the Thomas Domes, the load cells were selected for weighing by suspension so they could be mounted overhead. Special care has been taken to protect the load cells from a weight overload and from extreme environmental conditions which may be present inside of the Thomas Domes.

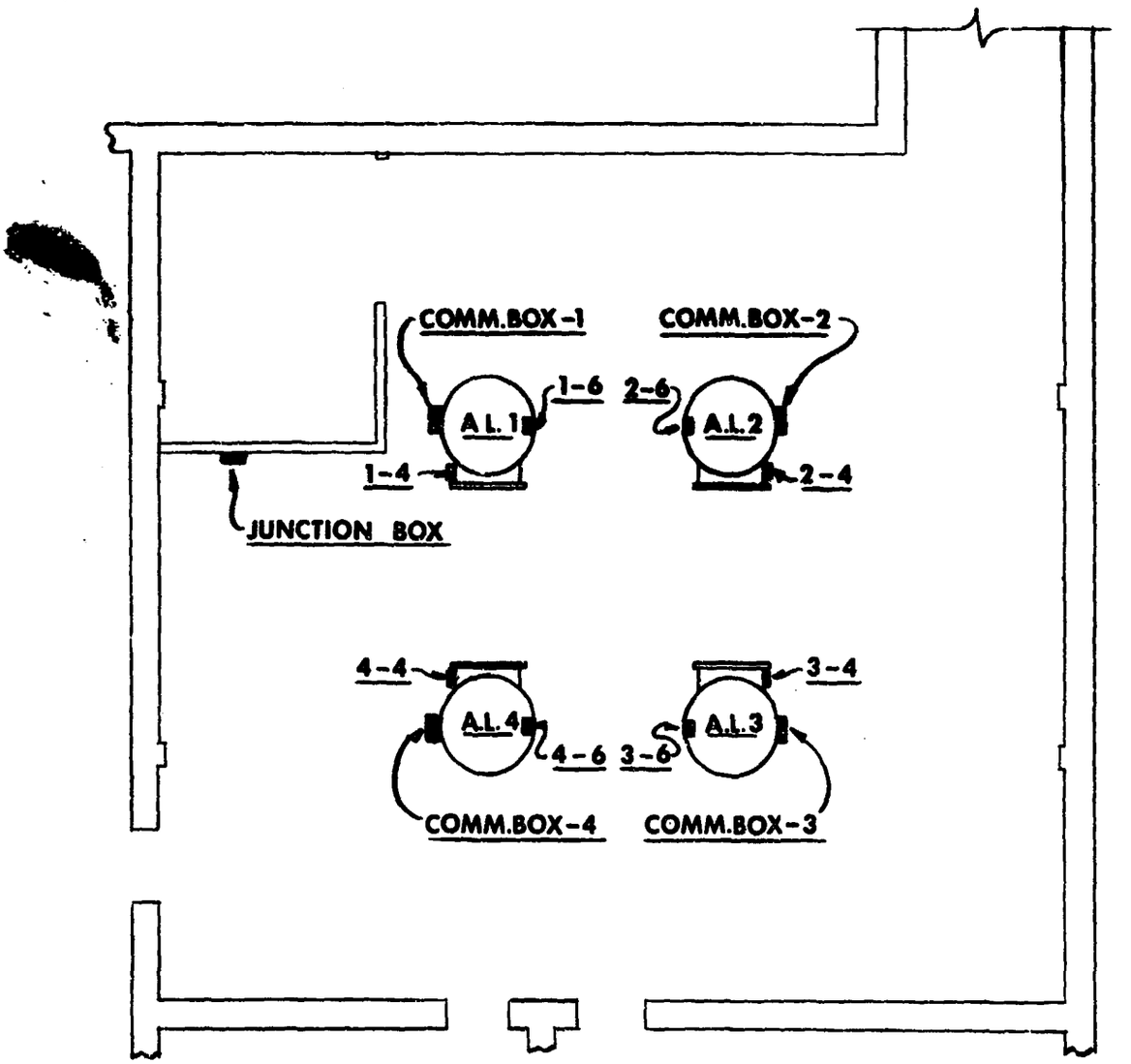
The main control panel is located in the northeastern corner of the main floor of Facility B. The console itself is divided into three sections as shown in figure 24. From left to right the first section (unit 1) contains four transducer switching units and auxiliary dome communications. The middle position (unit 2) contains two strain gage indicators and one transducer switching unit. The transducer switching unit in the middle section is for future use in the postexposure animal holding facility. The last section (unit 3) contains the data processing equipment which will be utilized for computer interfacing.



COMMUNICATION SYSTEM
MAIN FLOOR

Figure 20

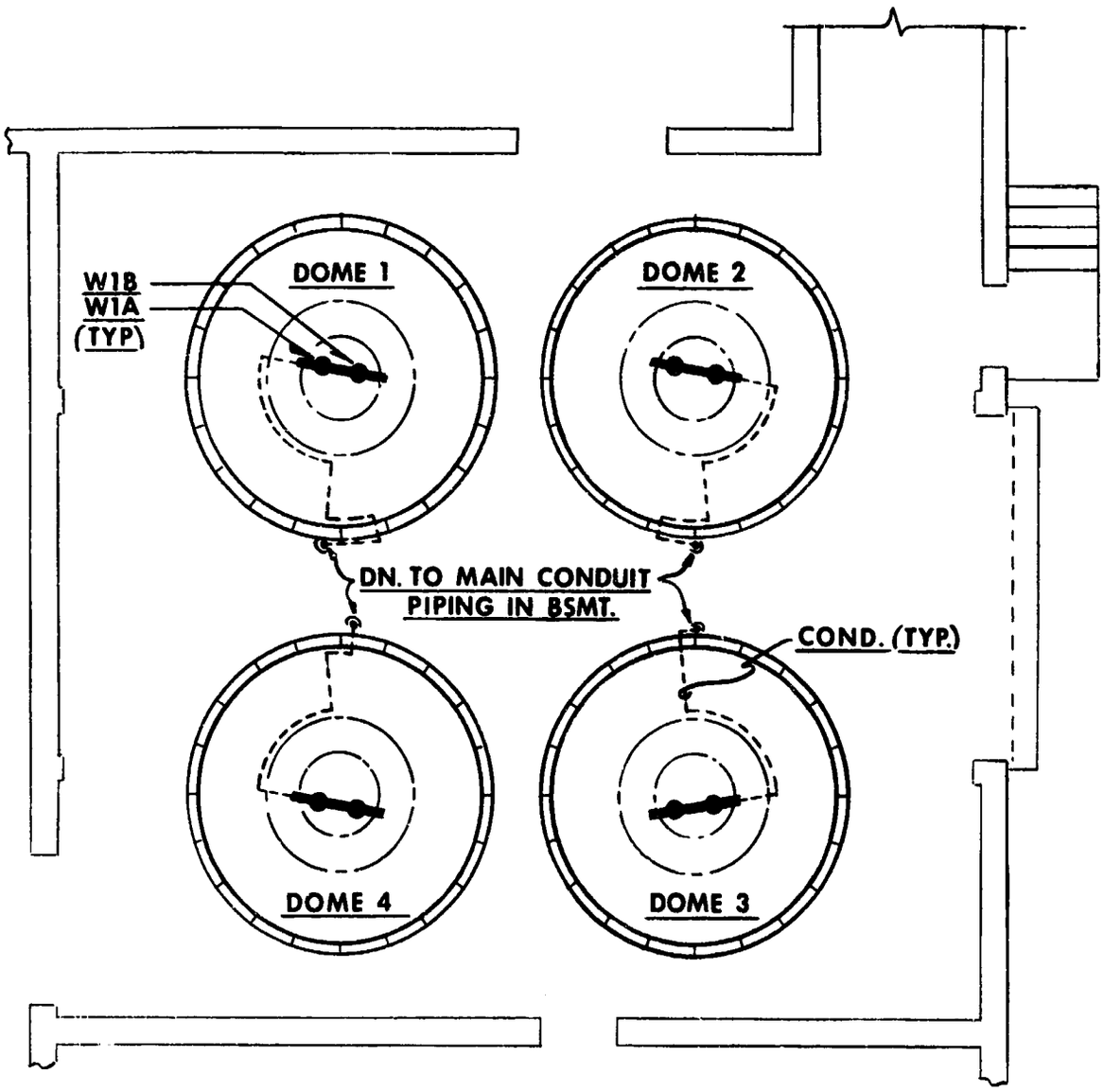
Communication System - Main Floor



COMMUNICATION SYSTEM
BASEMENT

Figure 21

Communication System - Basement

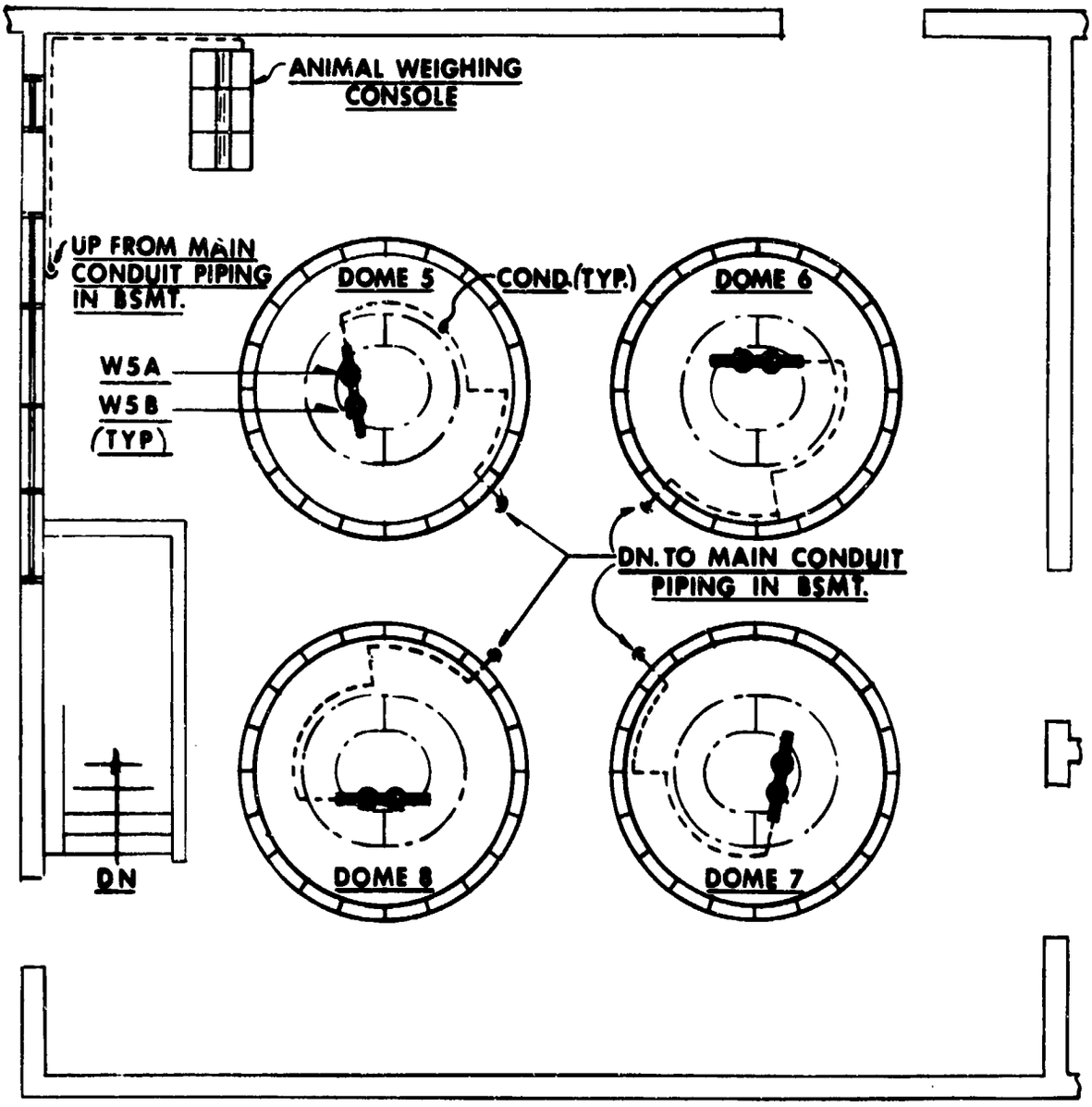


ANIMAL WEIGHING SYSTEM

FACILITY "A"

Figure 22

Animal Weighing System - Facility "A"

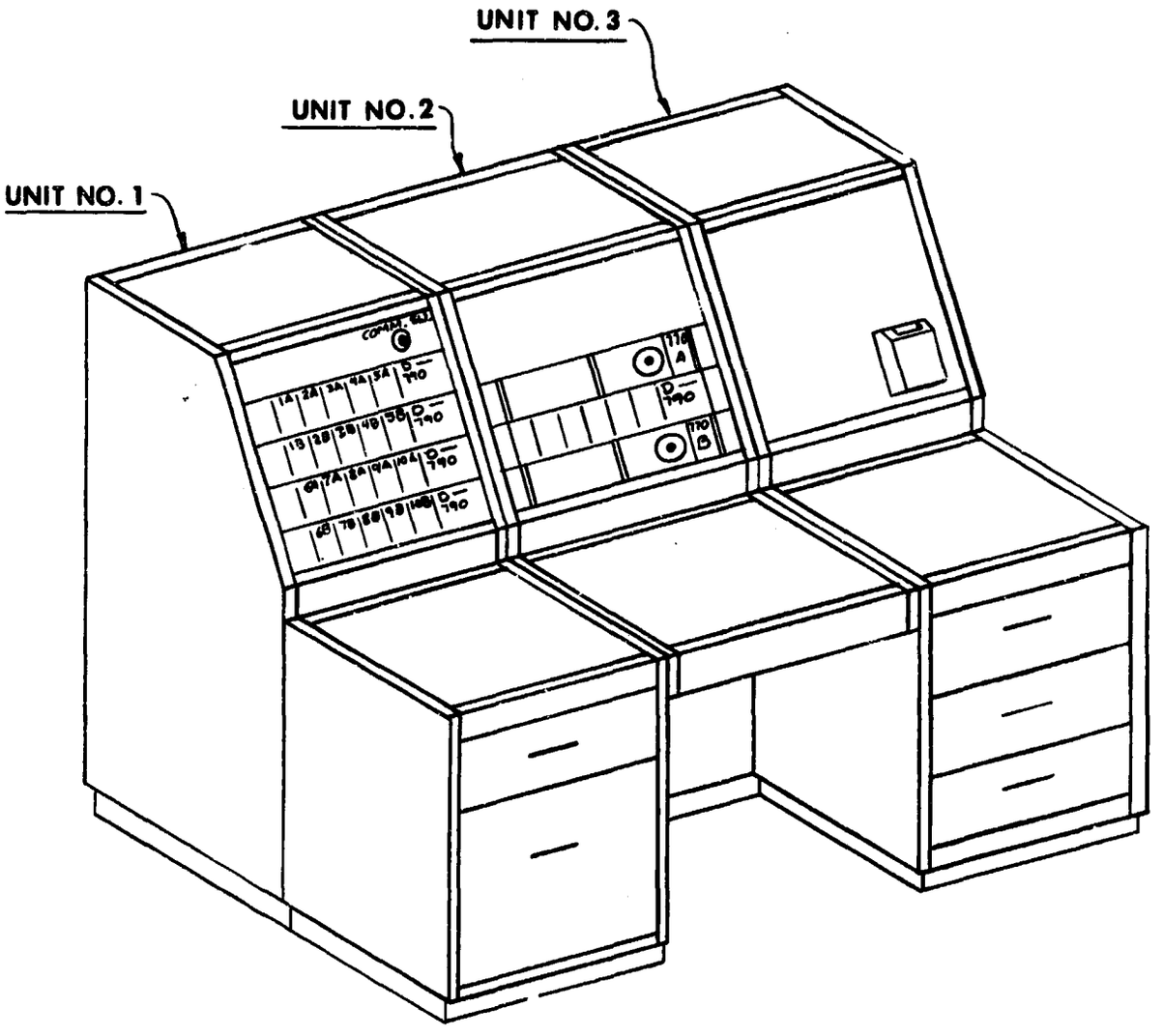


ANIMAL WEIGHING SYSTEM

FACILITY "B"

Figure 23

Animal Weighing System - Facility "B"



ANIMAL WEIGHING CONSOLE

Figure 24

Animal Weighing Console

An auxiliary communication station was installed in unit 1 of the console. This station is operated in conjunction with the main communication systems of Facility A or B of the Thomas Domes. The station is also provided with a communication cord which plugs directly into the headset and with a switch which connects the headset into any one of the nine altitude chambers. The time involved in animal weighing is minimal because of two design specifications. One damps out animal movement from the recorded weights, the other permits electronic calibration of the load cells after initial calibration with standard weights.

The accuracy and repeatability of the animal weighing system was tested at each station prior to actual routine use. Standard metal balance weights, either stainless steel or brass, were weighed in a random manner until each combination had been weighed 5 times. The results of this test for a typical dome weighing center are shown in table II for the 20 kg load cell used for large animals and for the 2000 gram load cell used for rodent weighing. Table III presents the results of weighings made with the same two load cells using living animals. The analysis of the data indicated that the 95% confidence limits of a single determination for the 20 kg load cell using inanimate weights are $\pm$ 10 grams and with living animals $\pm$ 100 grams. The 95% confidence limits for the 2000 gram load cell are $\pm$ 1 gram for either inanimate or living objects.

The installation of the new weighing system has resulted in improved reliability of experimental animal weighing within the domes.

Operation of Oxidizer Dilution Equipment

When the toxicity of a reactive oxidizer is to be determined by exposure of animals in chambers, it has been found desirable to use a dilute source of the oxidizer rather than a tank of the pure material. In this way, personnel conducting the experiment are not subject to exposure to high concentrations of toxic material in case a leak develops. It appears also that the reactivity of an oxidizer may be reduced on dilution so that there is a greater probability of delivering unchanged oxidizer into the chamber rather than some reaction product. Because of these considerations, an oxidizer dilution facility was constructed in Building 79A which is relatively isolated from the rest of the Toxic Hazards Laboratory.

Dilution of oxidizer compounds occurs in a room measuring approximately 6' x 15', separated from the rest of the building area by walls of steel-reinforced concrete block. Sheet steel duct work leading to a large blower forms the ceiling of the room. The room is essentially a walk-in fume hood and can remove fumes released through equipment malfunction, etc., so that personnel are not exposed to any toxic materials.

TABLE II

Repeatability of Weighing Results
with Metal Weights

20 Kg Load Cell

Weights Nominal	Test 1	Test 2	Test 3	Test 4	Test 5
0	0	0	0	0	0
5	5.01	5.01	5.01	5.02	5.01
10	10.02	10.02	10.02	10.03	10.02
15	15.02	15.02	15.02	15.02	15.02
20	20.00	20.00	20.01	20.01	20.00
19	19.01	19.01	19.01	19.01	19.01
18	18.01	18.01	18.02	18.02	18.02
17	17.01	17.01	17.02	17.02	17.01
16	16.01	16.01	16.02	16.02	16.01
14	14.02	14.02	14.02	14.03	14.02
13	13.02	13.02	13.02	13.03	13.02
12	12.02	12.02	12.02	12.03	12.02
11	11.02	11.02	11.02	11.03	11.02
9	9.02	9.02	9.02	9.02	9.02
8	8.02	8.02	8.01	8.02	8.01
7	7.01	7.01	7.01	7.02	7.01
6	6.01	6.01	6.01	6.02	6.01
4	4.00	4.01	4.01	4.01	4.01
3	3.00	3.01	3.00	3.01	3.00
2	2.00	2.00	2.00	2.01	2.00
1	1.00	1.00	1.00	1.01	1.00

2000 Gram Load Cell

Weights Nominal	Test 1	Test 2	Test 3	Test 4	Test 5
0	0	0	0	0	0
50	50	49	49	49	49
100	100	99	99	99	99
200	199	199	199	199	199
500	499	499	499	499	499
700	700	700	699	700	699
1000	1000	1000	1000	1000	999
1200	1200	1200	1200	1200	1199
1500	1500	1500	1500	1500	1500
1700	1700	1700	1700	1700	1700

TABLE III

Repeatability of Animal Weighing
with Load Cells

20 Kg Load Cell

Dog Number	Test 1	Test 2	Test 3	Test 4	Test 5	Average Weight
G39	9.52	9.53	9.52	9.49	9.48	9.51
G43	8.78	8.78	8.78	8.70	8.70	8.75
G41	7.76	7.75	7.73	7.66	7.66	7.71
G71	10.57	10.54	10.53	10.42	10.47	10.51
G75	9.92	9.91	10.02	9.90	9.96	9.94

2000 Gram Load Cell

Rat Number	Test 1	Test 2	Test 3	Test 4	Test 5	Average Weight
10	207	206	207	207	206	206.6
9	195	194	193	194	193	193.8
45	174	174	173	173	174	173.6
99	207	207	207	207	206	206.8
16	192	191	191	191	191	191.2

Oxidizer Dilution System

The oxidizer dilution system that was constructed in the walk-in hood is represented schematically in figure 25. The construction materials are of types compatible with most oxidizer applications. All transfer lines are 1/4" OD, 304 stainless steel. The valves used are either of the bellows type or of the packed type utilizing TFE Teflon® as the packing. Stainless steel, type 316, comprises the balance of construction material. While Teflon® is not universally recommended for dynamic oxidizer service (reference 39), preliminary experiments showed that Teflon® is acceptable for valve packing in a system with low oxidizer pressure (0-15 psia) and low flow rates (0-9 liters/min). The Bourdon tube pressure gauges are manufactured of 316 stainless steel, as is Tank D. Brass is used only in the commercially obtained HF trap and the valve on Tank D.

Two configurations of bellows valves were used in the dilution system. After oxidizer (Tank A) and the nitrogen fill (Tank C), preset bellows needle valves control the flow of oxidizer and nitrogen respectively. The remaining bellows valves are remotely actuated by an air supply from the control panel located outside of the hood, thus providing a safe means of operation during the more hazardous phases of the dilution process. The valve on the tank of pure oxidizer (Tank A) is operated from the control panel also, having a mechanical linkage through the blast wall for this purpose. The packed valves are hand operated from inside the hood only during relatively "safe" periods of dilution.

Two prepurified nitrogen tanks are included in the system. Tank C is commercially filled nitrogen tank (2500 psig) which acts as the oxidizer diluent supply. Nitrogen gas for purging and leak testing the entire system is supplied by Tank B, thereby leaving Tank C full for diluent purposes.

Either of two devices may be used to dispose of oxidizer remaining after passivation, after filling Tank D, or in case of emergency dumping of the tank of pure oxidizer. The first method is controlled mixing and combustion of the oxidizer with natural gas in a standard Terrill burner fitted with an additional burning stack. The second method involves passing the vented oxidizer through a hot charcoal burner. The hot charcoal reacts with most of the oxidizer and the resultant high temperature decomposes the remaining oxidizer (reference 47).

Removal of gases from the system is done by one of two methods. The purge gas is removed through an oil type vacuum pump which is preceded by a molecular sieve filter to prevent back diffusion of oil vapors. Excess oxidizer is condensed in the cryogenic trap at liquid N_2 temperatures and vented to either the Terrill burner or charcoal. The liquid nitrogen Dewar flask is automatically raised around the trap using controls at the control panel.

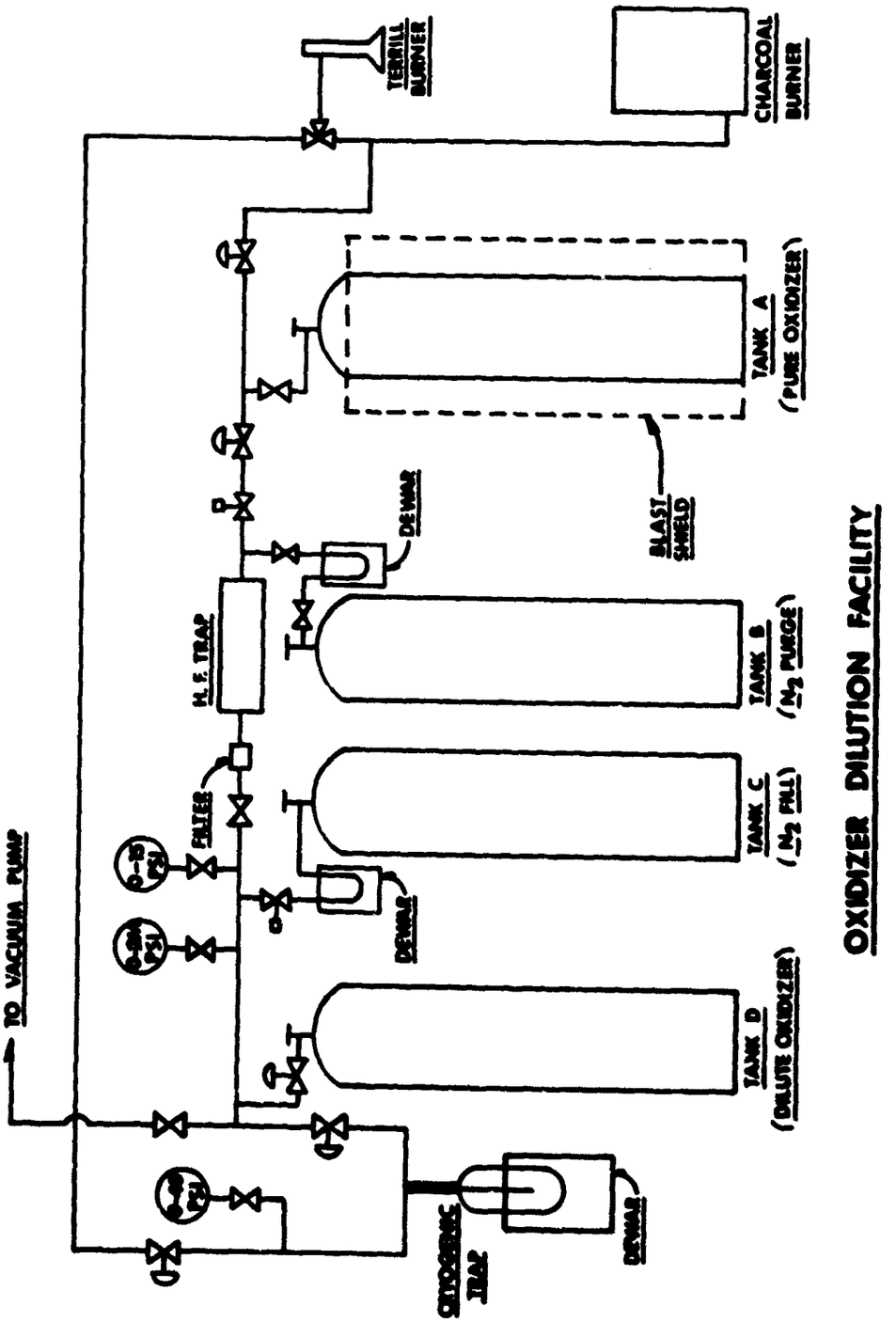


Figure 25

Oxidizer Dilution Facility

The dilution system is operated by combining a nitrogen purge of all lines and Tank D with a vacuum and pressure check of all fittings and valves. Tank D and the lines are evacuated, purged and passivated with the oxidizer. The cryogenic trap is then cooled to remove the oxidizer used for passivation which is then vented off after removal of the liquid nitrogen Dewar flask to one of the disposal systems previously mentioned. Next, the proper pressures of oxidizer and diluent are delivered into Tank D. A final purge of all lines with nitrogen is made before the system is sealed and Tank D removed for subsequent analysis by infrared spectroscopy.

Modifications of Oxidizer Dilution System

Two incidents occurred during early dilutions of oxygen difluoride which necessitated changes of the original dilution system. The original system included a corrosive gas regulator, which was constructed of monel and stainless steel with Kel-F seat and gasket, connected to the oxidizer tank (Tank A). During the initial opening of the source tank of OF_2, a burnout occurred in the regulator, causing pure OF_2 to be vented off in the hood. After shutdown procedures were completed, a burned out Kel-F gasket was discovered. The failure was due to one or more of three possible causes:

1. Contamination of the Kel-F gasket.
2. Contaminated OF_2 tank valve.
3. Inappropriate regulator design or materials.

In view of the uncertainty of the cause of the failure and the probability of a reoccurrence, the regulator was removed from the system.

The second incident occurred after purge of the cryogenic trap with nitrogen. A small amount of oxidizer was left in the trap prior to evacuation by the mechanical oil pump. It was felt that the oxidizer would be dilute enough so that the pump oil would not react with it. However, this was not the case. In rapid succession several small explosions occurred in the pump during evacuation of the trap.

Therefore, a relatively simple device was installed. The tube delivering purge nitrogen now extends halfway down the cryogenic flask through a hollow fitting. The device forces the flow of gas through the cryogenic trap instead of by-passing the opening at the top of the trap.

Since the modifications to the system were made, dilutions of oxidizer have been problem free. Tanks of 1% OF_2 have been supplied to the Laboratory Operations Section for use in exposure experiments. Present plans call for diluting ClF_3 under the same conditions as OF_2 for acute experiments scheduled to begin in the next year.

SECTION III

RESEARCH PROGRAM

The inhalation toxicology research program of the THRU covers a broad area of interest ranging from standard industrial hygiene toxicology problems to the more exotic but real problems of determining safe limits for continuous low level contaminant exposures in spacecraft atmospheres. The primary mission of the THRU program is to provide answers to these practical problems concerning the health not only of Air Force personnel but of the civilian population working with the same or related materials.

As in previous report periods, some of the research experiments discussed herein were initiated in the preceding year and some that were started this year will carry over into the next reporting period. Toxicity screening of space cabin construction material is a continuing project with individual experiments conducted whenever sufficient materials are made available for testing.

Beta Cloth Glass Fiber Dust

At the request of the National Aeronautics and Space Administration a series of experiments was conducted to evaluate the capability of Beta cloth dust to produce upper respiratory and nasal irritation or, more specifically, its ability to produce symptoms similar to the common cold. Beta cloth has been found useful in space flight programs, because of its nonflammability, for clothing and for flexible ties to hold other materials in place. The Beta cloth uniforms used for astronauts are similar to those used in the Thomas Domes for altitude work. They are woven from yarn consisting of bundles of glass fibers, approximately 2 to 3 microns in diameter and coated with a thin layer of Teflon®. In use, this type of garment can be abraded resulting in the production of very small fibrous glass dust particles well within the respirable range. In space flight small particles of Beta cloth dust can become suspended in the atmosphere indefinitely due to weightlessness and therefore may represent a potential source of respiratory irritation.

Inhalation exposures of animals to glass wool and insulation quality glass fibers were reported by Gardner (references 45 and 46) and by Schepers (references 41 and 42). These studies of large diameter (approximately 10-20 microns) fiber glass particles were somewhat conflicting and more comprehensive studies were later reported by Gross (reference 15) from which it was concluded that inhaled glass fibers were inert. These experimental studies with rodents were supported by the roentgenographic

findings on large numbers of fiber glass workers reported by Wright (reference 51). The results of this study demonstrated no distinctive x-ray shadow which could be interpreted as resulting from fiber glass dust exposure.

Technological advances have led to the manufacture of small diameter glass fibers which are useful as textiles. The Beta fiber is one of these new fibers.

Gross (reference 16) exposed rats by the inhalation route to the dust of Beta fibers both alone and with coating materials on a daily basis for over 1 year. Although his experiments were incomplete, his preliminary findings during the first 12 months of serial sacrifice indicated that it was biologically inert.

The larger glass fibers had been shown to produce skin irritation by Siebert (reference 43) and Salzberger et al. (reference 40). Further studies by Heisel et al. (reference 20) demonstrated that the skin effects are primary irritation and that sensitization did not occur in those test subjects studied.

The current investigation was conducted to determine if Beta fiber dust from Teflon®coated yarn could produce nasal or respiratory irritation in primates. Since actual space cabin exposures of men to the cloth dust could occur in conjunction with the presence of small amounts of other contaminants that could be irritating, the studies were conducted with Beta cloth dust alone and in the presence of either chlorine or 1,1,2-trichloro 1,2,2-trifluoroethane (Freon 113).

A series of preliminary experiments were conducted to determine a level of chlorine or Freon 113 concentrations one tenth of that which would produce toxic signs during continuous exposure for 7 days.

One to 4 rhesus monkeys were used in the 24-hour preliminary tests. Groups of 4 female monkeys (3-6 kg) were utilized in all other studies. Groups of 2 monkeys each served as controls and were exposed to air only. Exposures were conducted in Rochester Chambers, operated with 20 cfm nominal air flow and maintained at 70-75 F and 50-60% relative humidity. Exposures were continuous except for brief animal servicing periods each morning to replenish food and water.

All monkeys used in this study had been carefully examined for any signs of upper respiratory tract disease before exposure. Symptomatology was recorded every hour during the course of exposure. At the conclusion of a test run, the animals were again examined for signs of ocular, nasal and oral mucosa irritation, then lightly anesthetized with sodium pentobarbital and submitted for immediate gross pathologic examination of both the upper and lower respiratory tract.

Two separate 24-hour exposures, one week apart, of a male monkey to measured concentrations of 2040 and 3575 ppm Freon 113 produced no signs of irritating effects either during the exposure or at postexposure necropsy. 2000 ppm appeared to represent a suitable level for purposes of the 7-day test.

Four monkeys were exposed for this time period to a mean concentration of 1925 ppm Freon 113. No abnormalities were noticed during exposure. Gross pathology findings were negative.

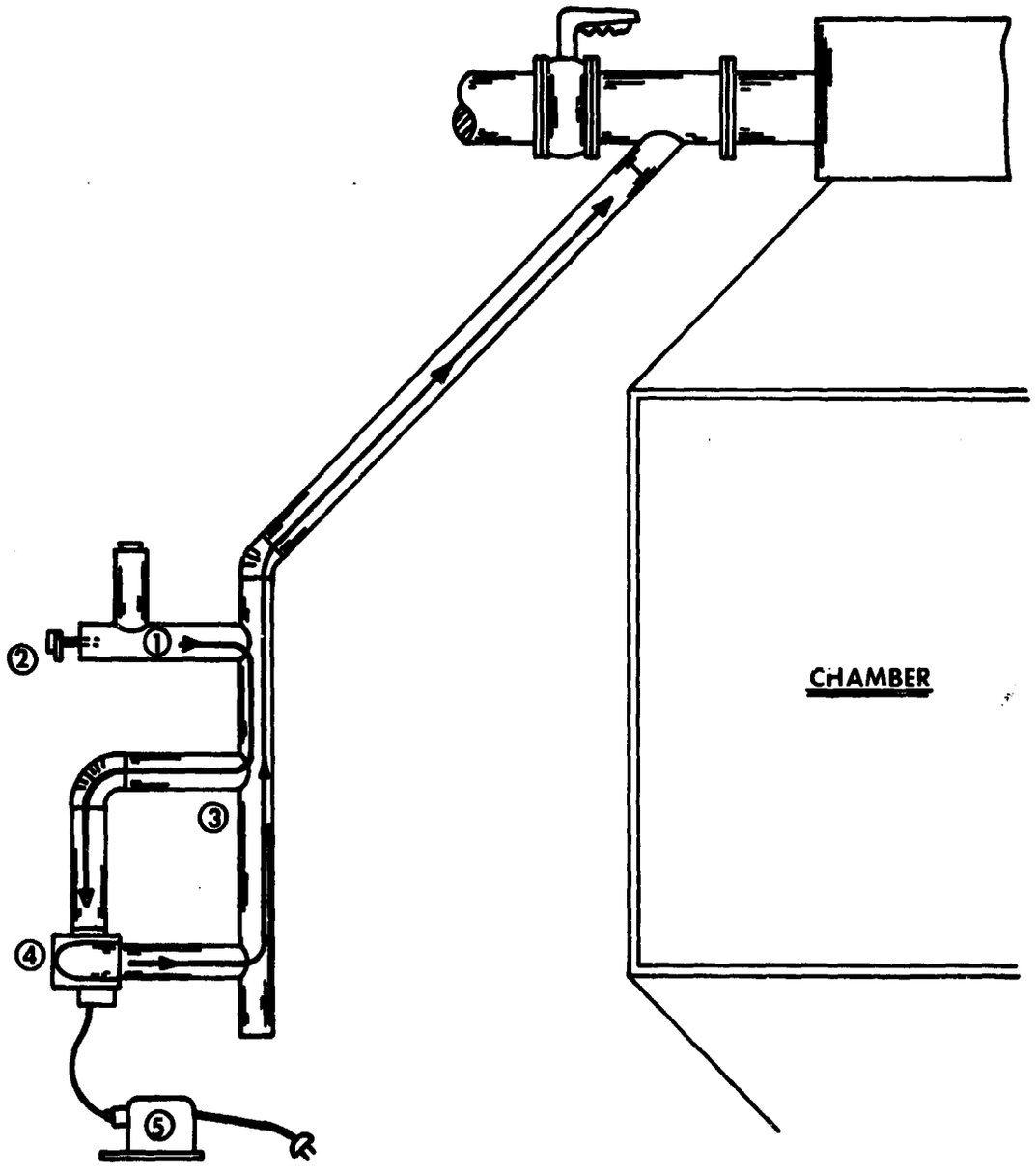
Definite signs of irritation were seen in monkeys exposed to selected concentrations of 5 and 1 ppm chlorine for 24 hours. The indications of irritation from the exposures, although differing in degree and onset, included lacrimation, salivation, emesis and frequent gasping. Gross examination of the respiratory tract showed hyperemia of the tracheal and bronchial mucosa.

On the basis of the preceding information, a dose level of 0.1 ppm Cl_2 was selected for the 7-day exposure. Accordingly, 4 monkeys were exposed to a mean concentration of 0.11 ppm for this time period. No symptoms indicative of irritation were observed during or immediately after exposure.

Beta fiber yarn coated with Teflon® was ground in a ball mill as a water slurry using 1 inch steel balls. After 5 days grinding, the slurry was cleaned by acid treatment to remove iron originating from the mill and balls; then filtered and washed. Subsequently, sedimentation techniques were used to collect particles in the 3-7 micron size range. The dust particles were then dried for use in animal exposures.

The dust exposures were generated using an air elutriator shown in figure 26. The reservoir (1) was filled with dust and replenished when necessary. A constant speed timing motor (2) coupled to a five-turn coiled rod produced a continuous delivery of glass fiber dust to the loop delivery system (3). Effluent air from the shaded pole blower motor (4) carried the dust fibers through the piping to the exposure chamber. A variable-voltage transformer (5) provided a means of controlling the blower motor over a wide range of speeds thus ensuring the capability to maintain the desired exposure chamber dust concentration of the required particulate size.

Continuous measurement of the chamber dust concentration was made with a dust photometer coupled to a variable speed recorder. This instrument measures the forward scattering of light from particulate matter drawn continuously through a dark field illumination chamber.



BETA CLOTH DUST GENERATOR

Figure 26

Beta Cloth Dust Generator

The dust exposure concentration was selected to be 15 mg/M³ which is the ACGIH threshold limit for nuisance dusts. The average actual chamber concentrations in the three experiments were as follows:

Run	Beta Cloth Dust	Chlorine	Freon 113
1	14.1 mg/M³	-------	---------
2	13.4 mg/M³	0.11 ppm	---------
3	12.7 mg/M³	-------	2100 ppm

Groups of 4 monkeys each were exposed continuously for 8-day periods to each of the above environments. The animals were observed hourly for signs of irritation throughout the exposure and their entire respiratory tracts examined at necropsy. There was no evidence of irritation from the Beta cloth fiber dust either singly or in combination with Cl_2 or Freon 113 at the levels tested. Histopathologic examination of the nasal passages and respiratory airways failed to show any differences between the exposed monkeys and their controls.

Methylisobutylketone (MIBK) Range Finding Study

Methylisobutylketone (MIBK) is one of the lower aliphatic ketones that have found wide use as solvents for or in many of the materials used in the spacecraft industry. Toxicity data were desired because of possible contamination in a closed loop life support system, due to off-gassing of this compound from Fluorel, a plastic finding increasing use. There are very limited toxicity data on the aliphatic ketones with the exception of acetone. They are thought to be relatively nontoxic but are known to be central nervous system depressants at high concentrations. These tests were designed to determine a biological effect level of inhaled MIBK, under continuous exposure conditions in order to establish criteria for subsequent long-term studies.

Rats, mice, dogs and monkeys were continuously exposed to a mean concentration of 100 ppm MIBK for two weeks at ambient conditions in the Thomas Domes. The number of test animals used were 4 monkeys, 8 dogs, 40 mice, and 50 rats. Three monkeys, 4 dogs, 20 mice, and 25 rats served as controls and were placed in another Thomas Dome under the same conditions with the exception of contaminant. One monkey in each group had implanted cortical electrodes for evaluation of CNS effects.

The MIBK used in this study was purchased from the Matheson Company, Inc., East Rutherford, New Jersey. A gas chromatographic method, described on page 6, was used for continuous monitoring.

The contaminant generating system was controlled by a dual syringe feeder. A measured amount of liquid MIBK was evaporated in air and introduced into the dome air supply line. This system provided adequate control over the contaminant concentrations for the 2-week exposure period.

A series of test programs were designed to evaluate the inhalation effects of the MIBK exposure as outlined below:

Preexposure Tests

1.	Body Weight	Biweekly
2.	Clinical Chemistry	(SMA-12)
3.	Hematology	(Biweekly)
4.	EEG	24 hour preexposure

During Exposure Tests

1. Activity Measurement
2. Symptomatology
3. Mortality Response

Postexposure Tests

1. Body Weights
2. Organ to Body Weight Ratios
3. EEG
4. Clinical Chemistry
5. Hematology
6. Pathology
7. Blood pH and Gases
8. Urinary 17-Ketosteroids
9. Serum 17-Ketosteroids

There were no signs of toxic response during exposure. At the end of the 2-week exposure period there was no difference in cortical activity between the exposed monkeys and controls nor were any significant differences observed in hematologic or clinical chemistry measurements for either dogs or monkeys. Gross pathologic examination of tissues from both exposed and control animals failed to reveal any apparent

differences. Histopathology examinations were not conducted although tissues were saved for possible future reference. Blood gas measurements made on dogs did not show any effects attributable to MIBK exposure.

Organ weight and organ to body weight ratios were evaluated and the kidneys found significantly altered in rats apparently due to the MIBK exposure as shown in table IV.

TABLE IV

Effect of Inhaled Methylisobutylketone (100 ppm) on Organ Weights of Albino Rats

	Heart	Lung	Liver	Spleen	Kidneys
EXPOSED					
N	50	48	50	50	50
$\bar{x}$ Organ Wt.[1]	1.0	1.2	8.6	0.8	1.7**
$\bar{x}$ Ratios[2]	0.416	0.547	3.756	0.353	0.729**
CONTROLS					
N	25	23	24	25	25
$\bar{x}$ Organ Wt.[1]	0.9	1.3	8.4	0.8	1.5
$\bar{x}$ Ratios[2]	0.417	0.569	3.753	0.346	0.670

[1] grams
[2] grams/100 grams body weight
** significant at 0.01 level

Since the only indication of a toxic response to the 2-week inhalation exposure of 100 ppm MIBK was its effect on rat kidney weight and a slight indication of depressed growth in rats, a second 2-week exposure was conducted at an atmospheric concentration of 200 ppm MIBK, and the same biological measurements and examinations were performed.

The animals exposed to 200 ppm MIBK showed no outward toxic effects that could be attributed to the exposure. In exposed rats liver and kidney weights were statistically different from those in the control group as shown in table V.

TABLE V

Effect of Inhaled Methylisobutylketone (200 ppm)
on Organ Weights of Albino Rats

EXPOSED

	Heart	Lung	Liver	Spleen	Kidneys
N	50	46	50	50	50
$\bar{x}$ Organ Wt.	0.9	1.3	9.0**	0.8	1.8**
$\bar{x}$ Ratios	0.357*	0.499	3.445**	0.291	0.694**

CONTROLS

N	50	42	50	50	50
$\bar{x}$ Organ Wt.	0.9	1.3	8.2	0.8	1.5
$\bar{x}$ Ratios	0.343	0.510	3.198	0.303	0.582

* significant at the 0.05 level only
** significant at the 0.01 level

From the data obtained in this experiment and observations made from the previous MIBK inhalation study, it would appear that the kidney is the major target organ challenged by the exposure to MIBK. Physical properties of MIBK (bp 117 C) suggest that the kidney may be the major route of excretion. The persistent finding of significant changes in kidney weights and kidney to body weight ratios in the rat did indicate that MIBK exposure may be causing kidney changes. Histopathologic examination of the rat kidney revealed toxic nephrosis in the proximal tubules of MIBK exposed rats. Subsequent examination of the kidneys of the rats previously exposed to 100 ppm MIBK confirmed these results.

The results of the 2-week exposures to MIBK suggest that the 100 ppm level is a satisfactory dose for use in a long-term study. A 90-day continuous exposure of the 4 animal species has been scheduled for initiation in the next year and the results will be discussed in ensuing reports.

Inhalation Toxicity Studies with 1,1,2-Trichloro-1,2,2-trifluorcethane (Freon 113)

Freon 113 is one of the halogenated ethanes with wide industrial application as a refrigerant, solvent, and cleaning agent. Toxicity studies have shown it to be relatively nontoxic, and the American Conference of Governmental Industrial Hygienists has assigned a threshold limit value (TLV) of 1000 ppm.

The metabolic fate of Freon 113 has not been established, and it is not known whether the toxic symptoms seen are due to the unchanged compound or one of its metabolic products. Some of the acute toxic manifestations have been characterized in man and laboratory animals as being due to effects on autonomic and central nervous systems.

This study was designed to determine a biological effect level for inhaled Freon 113 under continuous exposure conditions for use in subsequent long-term experiments. The exploratory exposure concentration chosen was 2000 ppm for a period of 14 days.

The experimental and control groups of animals were comprised of 4 monkeys and 8 dogs in each group; 40 mice and 50 rats were used in experimental groups, and control groups consisted of 20 mice and 25 rats. Monkeys and dogs were females while rats and mice were males. The exposure was conducted in a Thomas Dome at ambient conditions.

The "Freon 113 TF" used in this study was purchased from the E. I. DuPont De Nemours Company, Inc., Wilmington, Delaware. Freon 113 contaminant analysis was made by gas chromatography as described earlier in this report.

The changes observed in animals exposed to 2000 ppm of Freon 113 for 14 days were all minimal, and could not be related to the toxic effects of the compound. Enlarged thyroid glands were observed in all rhesus monkeys exposed. Rat kidneys were the only organs showing an increase in weight over control values. These differences were minimal, and could not be conclusively attributed to the exposure.

Toxicity of Spacecraft Materials

During the past year, a total of nine 7-day experiments exposing rodents to the gas-off products of Apollo spacecraft materials were carried out. The 9 experiments tested 121 materials in groups of 10-14. Three exposures to the gas-off products from larger groups of spacecraft materials for 60 days were performed. These 3 groups had 202 materials some of which had been subjected to 7-day tests in the preceding year. Another 60-day experiment utilizing 66 materials was begun but had not yet terminated at the end of the report period.

The animals were exposed in a closed-loop life support system which has been described by Johnson (reference 26).

Weighed portions of each material were placed in the oven of the life-support loop, except where material was limited or where solvent made up the major portion of the as-is substance. In the latter eventuality, a weight of as-is material was evaporated at room temperature until it appeared free of solvent; it was again weighed and placed in the oven of the life-support loop with the other test materials. At the conclusion of the experiment, the samples were reweighed and percent loss or gain calculated. Ten-gram portions were used for the 60-day studies and 100 grams for 7-day exposures.

Twenty male rats and 25 male mice were exposed to the combined gas-off products of all the materials in the oven which was held at 155 F. The system atmosphere was 100% O_2 at 5 psia pressure. Control animals were housed in a similar loop under the same experimental conditions except for contaminant. All animals were observed and weighed one week prior to exposure and at weekly intervals for four weeks postexposure. Necropsies were performed on half of the experimental animals two weeks postexposure and on the remainder four weeks postexposure. At necropsy, rat hearts, lungs, livers, spleens and kidneys were weighed and the organ to body weight ratios calculated. All animals were examined grossly and tissue samples taken for histopathological examination if some indication of toxicity became evident.

Samples of the chamber atmospheres and of condensed water from the loop chillers were taken for gas chromatographic analysis. Usually 5 to 7 components gassed off from each mixture of material in sufficient quantity to be identified. Known compounds with retention times approximating those of the gas-off products were selected as standards. Using these standards, apparent concentrations in the atmosphere and in the water were determined. Maximum concentrations of individual gas-off products rarely exceeded 50 ppm except for methane and were achieved

between the first and third day after the start of the exposure. A typical gas-off pattern for methane is shown in figure 27 for Group Z Apollo materials. By the seventh day, concentrations had usually decreased to insignificant values, and this was invariably the case by the fourteenth day. As might be expected, water condensate samples generally gave fewer peaks than the air samples, but followed the same pattern of increase and decrease.

In all the experiments performed during this report period, no indications of toxic effect by any of the gas-off products were found after exposures of 7 or 60 days.

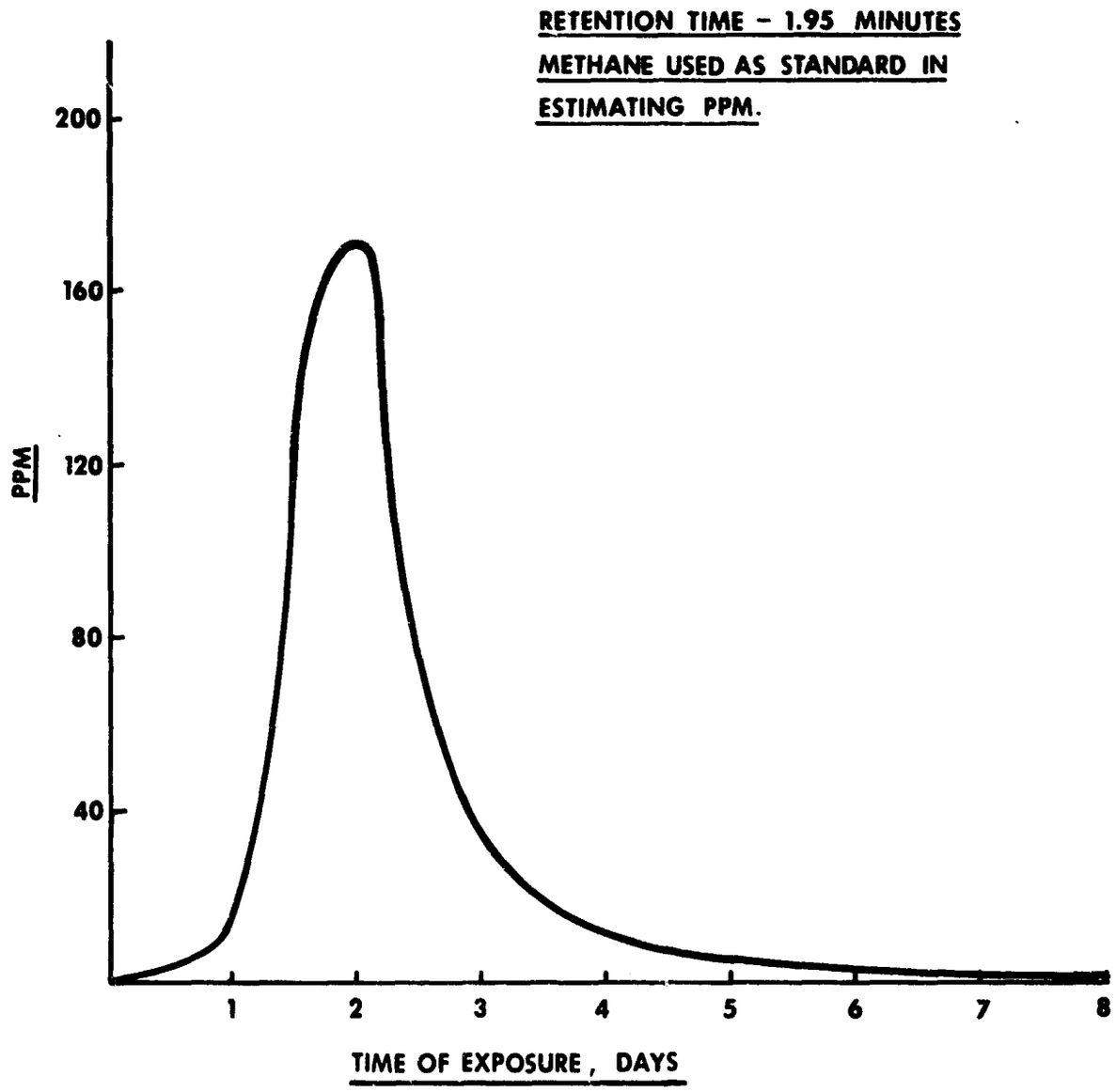
Chlorine Trifluoride (ClF_3)

Chlorine trifluoride is a high energy oxidizer that has demonstrated good potential for use in rocket propulsion systems. As the potential use of a new chemical expands so does the need for comprehensive toxicological data for establishing safe working exposure limits for personnel handling the material.

ClF_3 is a highly reactive compound with strong oxidizing properties approaching that of fluorine itself (reference 2). This compound, characterized by Grisard (reference 14), has been successfully used as a fluorinating agent in numerous reactions which customarily require elemental fluorine (reference 38).

One of the earliest studies on the toxicity of ClF_3 was made by Horn and Weir (reference 24). Chronic inhalation studies were made on dogs and rats exposed to sublethal concentrations of the gas for periods up to six months. Acute effects of ClF_3 on rodents have been reported by Dost et al. (reference 8).

Because of the exceedingly difficult problems associated with generating and monitoring ClF_3 in a dynamic system, many approaches were taken to stabilize the concentrations in the exposure chamber. One of these measures consisted of passivating the exposure chamber with the pure compound. By this method, our efforts were finally successful in obtaining a stable concentration and consequently a standard curve was made. Some of the problems associated with the reactive nature of this compound were reported by Dost et al. (reference 7). It hydrolyzes readily in ambient air to HF and products yet unidentified (reference 37). Relative humidity increased the rate of decomposition.



RELATIVE GAS-OFF PATTERN OF CONTAMINANT NO. 1
GROUP Z

Figure 27

Relative Gas-Off Pattern of Contaminant No. 1
Group Z

Chlorine trifluoride was purchased from the Matheson Company, Inc., East Rutherford, New Jersey. The analytical procedure developed to measure the concentration was based on the reaction of ClF_3 or its decomposition products with dimethylamine as previously described.

The gas was generated through a stainless steel delivery system to a 30 liter glass chamber coated with Teflon®. This system was used to expose rodents; descriptions of the chambers used have been given in previous reports (reference 29). The inhalation chamber for large animals was constructed of materials specified to be compatible with the compound.

Numbers and sex of animals used in these studies were: 4 rhesus monkeys at each exposed level, males and females; 8 male Wistar rats, and 15 male ICR mice.

Observations for toxic signs were made during the exposure and for a period of 14 days postexposure. Deaths occurring within this time period were also used to calculate the mortality response. Gross pathology examinations on a representative number of animals exposed to lethal and sublethal concentrations were also performed.

Clinical signs of toxicity observed in animals exposed to ClF_3 were typical of exposure to tissue irritants. Lacrimation, salivation, dyspnea, and rhinorrhea were the most common symptoms seen during the exposure in rats and mice. Survivors often developed bloody discharges from eyes and nares, a few hours after the exposure, that lasted for several days. Symptoms observed in monkeys included evidence of bronchotracheal, and gastrointestinal mucosal irritation as seen by the sneezing, coughing, and gagging reflex. Animals exposed to lethal concentrations demonstrated a general paresis, labored breathing, and a cyanotic appearance which usually preceded coma and death.

Table VI represents the mortality responses obtained from 60-minute exposure to ClF_3. Most of the deaths occurred two to three hours after the exposure (no deaths occurred during the exposure). Delayed deaths were recorded up to 36 hours postexposure.

Clinical chemistry determinations were made on pooled rat blood at 24 hours and 7 days postexposure. There were no significant differences found. Methemoglobin determinations were performed immediately and 24 hours postexposure. Even though the animals were cyanotic in appearance, there was no methemoglobin formed from these exposures.

TABLE VI

Acute Toxicity Response to Inhaled
Chlorine Trifluoride, 60-Minute Exposure

Species	Number Exposed	Concentration (ppm)	Mortality Ratios
Monkeys	4	127	0/4
Monkeys	4	150	2/4
Monkeys	4	200	1/4
Monkeys	4	300	2/4
Monkeys	4	400	4/4
Rats	8	200	0/8
Rats	8	400	6/8
Mice	15	125	0/15
Mice	15	150	2/15
Mice	15	175	4/15
Mice	15	200	14/15
Mice	15	400	15/15

TABLE VII

Comparative Chlorine Trifluoride LC_{50} Values
for Various Species

Species	LC_{50} Values (ppm)	95% Confidence Limits (ppm)
Monkeys	230	167-317
Rats	299	260-344
Mice	178	169-187

Animals dying from the acute effects of ClF_3 were examined immediately after death. All other examinations were made 14-days post-exposure. Lethal concentrations of ClF_3, in all species examined, produced massive alveolar and interstitial hemorrhage involving the entire lungs. Animals exposed to near lethal concentrations demonstrated congestion, edema, hemorrhage, and emphysema. These findings were often localized to discrete areas of the lungs. The degree of pathologic change correlated with the contaminant concentration. In a group of rats exposed to a lethal concentration, congestion was seen in most organs. These experiments have shown that ClF_3 and/or its decomposition products act primarily as lung irritants as demonstrated by the massive lung hemorrhage and edema.

Chlorine trifluoride is not a methemoglobinemia producing agent in monkeys. Dost et al. (reference 8) had reported this finding in rodents exposed to ClF_3. The cyanosis which frequently occurred at all levels of exposure was attributed to deoxygenation of hemoglobin resulting from decreased ventilation capacity and stasis in peripheral blood vessels.

The pattern of symptomatology and the course of delayed deaths in animals exposed to ClF_3 was quite typical of a strong tissue irritant, and resembled the effects of the pyrolysis products of $CBrF_3$ (reference 19). The LC_{50} values determined for the pyrolysis products of $CBrF_3$ were found to correlate well with the amount of HF formed in the reaction. Since it is highly probable that the principal hydrolysis product of ClF_3 is HF (3 moles per mole of ClF_3 reacted) a series of HF exposures were scheduled for comparative purposes. It also appeared that if this were true a more comprehensive understanding of HF toxicity would be necessary to establish safe exposure limits for ClF_3.

Hydrogen Fluoride

These studies were initiated to determine whether the toxic responses observed in monkeys, rats, and mice exposed to chlorine trifluoride (ClF_3) were the results of ClF_3 per se, or from one or more of its hydrolysis products. Extensive information has been published concerning the physical properties of HF but there is very limited information on its toxic properties. One of the earliest reviews on its inhalation toxicity was reported by Machle et al. (reference 34). They were able to show that HF was lethal to rabbits and guinea pigs exposed for 5 minutes to 1.0-1.5 mg/L (1,223 ppm to 1,744 ppm). Stokinger (reference 44) studied the acute and chronic effects of the compound on various species of animals--dogs, rabbits, guinea pigs, and rats. He was one of the first to indicate the possibility of obtaining different toxic responses from the various molecular aggregates of HF at room temperature. At room temperature, molecular aggregation of HF varies with slight changes in temperature and pressure. At elevated temperatures (100 C), the compound is in a monomolecular form and remains unaggregated at reduced pressure and temperature.

Hydrogen fluoride used in these experiments was purchased from the Matheson Company, Inc., East Rutherford, New Jersey. The vapor was metered from a cylinder wrapped with heating tape to maintain a temperature of 100 C preventing polymerization of the vapor. Gas flow was measured by a mass flowmeter. Concentration of HF in the exposure chamber was based on nominal calculated values.

Rhesus monkeys (male and female), Wistar rats (male), and ICR mice (male) were used in these tests. Numbers of animals used at each exposure level consisted of 4 monkeys, 8 rats, and 15 mice. The animals were placed in the exposure chamber and the desired concentration of HF was generated for 60 minutes. Toxic symptoms were recorded during the exposure and for a period of two weeks postexposure. Mortalities were recorded for a period of two weeks after which the animals were sacrificed and major organs examined.

The common signs of toxicity in all species were similar to those seen in ClF_3 exposures including excessive salivation, lacrimation, and nasal discharge. At lethal concentrations there were signs of severe respiratory distress and general paresis. In monkeys, gagging, sneezing, and vomiting were also seen at all exposure levels. Salivation, lacrimation, and paresis were more often seen at lethal concentrations. Postexposure findings included first and second degree skin burns which healed after several days. Respiratory distress and labored breathing was seen in animals surviving concentrations greater than the LC_{50} value.

Table VIII represents the mortality responses obtained from 60-minute exposure. Most deaths occurred within 72 hours postexposure. A few rodent deaths at the lethal concentrations occurred during the exposures. Characteristic LC_{50} values obtained by probit analysis are given in table IX.

The animals that died during exposure were immediately necropsied and examined microscopically for acute effects on the major organs. Massive lung hemorrhage and edema were characteristic findings. There was a direct relationship shown between the severity of pathology and the concentration of HF in the exposure chamber.

Monkeys and rats exposed to concentrations greater than the respective LC_{50} values and surviving for the 14-day postsacrifice period, showed lung congestion, edema, emphysema and diffuse hemorrhagic changes. Moderate to severe liver congestion was observed in exposed monkeys. Tracheal mucosal congestion was a frequent finding at all levels of exposure.

TABLE VIII

Acute Toxicity Response to Inhaled
Hydrogen Fluoride, 60-Minute Exposure

Species	Number Exposed	Concentration (ppm)	Mortality Ratios
Monkeys	4	690	0/4
Monkeys	4	1035	1/4
Monkeys	4	1575	0/4
Monkeys	4	1600	0/4
Monkeys	4	1750	3/4
Monkeys	4	2000	3/4
Rats	8	480	0/8
Rats	8	960	2/8
Rats	8	1440	5/8
Rats	8	2160	7/8
Rats	8	2650	8/8
Mice	5	500	3/5
Mice	5	550	3/5
Mice	5	600	5/5

TABLE IX

Comparative Hydrogen Fluoride LC_{50} Values
for Various Species

Species	LC_{50} Values (ppm)	95% Confidence Limits (ppm)
Monkeys	1774	1495-2105
Rats	1276	1036-1566
Mice	501	355-705

It appears from these studies and the works of others that the acute toxic effect of HF exposure is directly related to the degree of damage to the pulmonary system.

Symptomatology and gross pathological findings were similar in both compounds. There were differences in mortality responses (LC_{50}) shown in table X.

TABLE X

Comparison of Hydrogen Fluoride and Chlorine Trifluoride Acute Toxicity

	Hydrogen Fluoride (HF)	Chlorine Trifluoride (ClF_3)	
Species	LC_{50} (ppm)	LC_{50} (ppm)	Equivalent Molar HF
Monkeys	1774	230	690
Rats	1276	299	897
Mice	501	178	534

It appears from the LC_{50} data that the toxic response to ClF_3 was not due entirely to HF in monkeys but seems to be the major toxic factor for rodents. It has not been established, however, what compound (or compounds) might have contributed to this increased toxicity in monkeys.

The symptoms observed in each of the species indicate that HF is a respiratory irritant. Mortality from acute inhalation is due to lung damage. Mice are more susceptible to HF than rats, and monkeys are the least sensitive of the species tested.

Hydrogen fluoride concentrations were based on nominal calculated values. Since these exposures were conducted, fluoride electrodes (reference 12) have been used to measure HF in the same exposure chamber under similar flow conditions. Comparison of data shows excellent agreement between the measured and calculated values. In addition, the results obtained for the 60-minute LC_{50} in rats were in close agreement with the work of Carson et al. (reference 6) who reported a value of 1307 ppm as compared with 1276 ppm in this study.

Oxygen Difluoride

An important member of the family of fluorine containing gaseous oxidizers is OF_2 which has been reported to be a severe pulmonary irritant. Rodent exposures to as little as 10 ppm for 10 minutes have been shown to cause death (references 27 and 28). Death results from asphyxiation subsequent to severe pulmonary edema and hemorrhage. The odor of OF_2 resembles garlic and is perceptible somewhere between 0.1 and 0.5 ppm. Since a TLV for this gas has been established at 0.05 ppm, its odor is thought to be a safe warning property. There have been reports of OF_2 exposure to research chemists at three different industrial plants. All of these people were well aware of the hazard of breathing OF_2 and when the odor was noticed, they immediately left the exposure area. Each of the men exposed to OF_2 complained of soreness of the chest which disappeared within three days with no further effects. The estimated exposure levels were below 10 ppm in each case.

A human exposure to OF_2 investigated by THRU personnel was described in the last annual report in which it was concluded that the subject had been briefly subjected to an air concentration of approximately 1000 ppm. His survival and complete recovery was inconsistent with the toxicity data derived from rodent inhalation exposures and, therefore, more comprehensive studies on OF_2 toxicity were undertaken in the THRU laboratory. These studies are currently in progress and will be continued but a preliminary review of the results is worthwhile at this time.

Oxygen difluoride was diluted in dry nitrogen in the oxidizer facility previously described. Large cylinders of approximately 1% OF_2 in nitrogen were pressurized at 1000 pounds and analyzed for precise concentration. The dilute OF_2 was then introduced into the exposure chamber air stream for animal toxicity studies. The OF_2 gas was diluted primarily as a safety precaution due to its reported extremely high toxicity. Consequently, an entire safety procedure for handling of the OF_2 and exposure of animals was developed. The exhaust OF_2 from the exposure chambers was reacted with caustic in a scrubbing tower before discharge to the outdoors to prevent air pollution hazards.

Monkeys, dogs, rats, and mice were exposed to various concentrations of OF_2 for 60 minute periods and the LC_{50} value for that time period established. Table XI presents the mortality response observed in these 60-minute exposures. The comparative 60-minute LC_{50} values for the various species tested are presented in table XII along with the 95% confidence limits.

TABLE XI

Acute Toxicity Response to Inhaled
Oxygen Difluoride, 60-Minute Exposure

Species	Number Exposed	Concentration (ppm)	Mortality Ratios
Monkeys	4	16.0	0/4
Monkeys	4	21.0	1/4
Monkeys	4	32.0	3/4
Dogs	4	8.2	0/4
Dogs	4	16.0	2/4
Dogs	4	21.0	1/4
Dogs	4	32.0	4/4
Rats	10	2.2	0/10
Rats	15	3.0	14/15
Rats	10	4.2	10/10
Mice	15	1.0	5/10
Mice	15	2.2	8/15
Mice	15	4.2	15/15

TABLE XII

Comparative Oxygen Difluoride LC_{50} Values
for Various Species

Species	LC_{50} Values (ppm)	95% Confidence Limits (ppm)
Monkeys	26.0	17.0-42.0
Dogs	26.0	16.0-43.6
Rats	2.6	2.5-2.7
Mice	1.5	1.2-2.0

The acute toxic response seen for rodents is consistent with previously reported data. However, there is an apparent difference between rodents and larger animals in the acute toxic response which may be a function of size of the exposed subject and may help to explain why humans have survived accidental brief exposures to high concentrations of OF_2.

In rodents the common clinical sign of toxic response is respiratory distress; in dogs and monkeys, gagging and emesis occurs followed by general paresis. Animals of all species that died from exposure to OF_2 had severe lung congestion, edema, and hemorrhage.

Induction of OF_2 Tolerance

A short study was run to determine whether preexposure of mice to sublethal doses of OF_2 would afford some protection to subsequent lethal concentrations of OF_2. Accordingly, three groups of 10 mice were exposed to a sublethal concentration of 1 ppm for 60 minutes. At three selected postexposure time periods, one preexposed and one untreated group were exposed to a nominal lethal concentration of 4 ppm for 60 minutes. The results are shown in table XIII. On the basis of the 3 time periods tested, the data indicated that tolerance is induced within 24 hours, maximizes at 8 days, and is still effective after 24 days.

TABLE XIII

Induction of Tolerance to OF_2 in Mice by Preexposure to 1 ppm

Group	Measured Concentration - ppm	Post-Treatment Time	% Mortality
Untreated	3.45	----	100
Preexposed	3.45	24 hours	60
Untreated	4.25	----	100
Preexposed	4.25	8 days	10
Untreated	3.50	----	100
Preexposed	3.50	24 days	50

Monomethylhydrazine, 6-Month Chronic Toxicity Study

The increased use of MMH as a rocket fuel suggested the need for reevaluation of the current threshold limit value of 0.2 ppm established by the ACGIH by analogy with hydrazine and unsymmetrical dimethylhydrazine. Previously reported results of acute and emergency exposure limit studies performed in this laboratory (references 18 and 32) provided a basis for the selection of appropriate dose levels for use in repeated inhalation studies. These tests were undertaken to determine the biological response of 4 animal species to repeated daily exposures to 2 and 5 ppm MMH for a 6-month period. The experiments are currently in progress in the Thomas Dome chambers.

Both experimental groups as well as the control set of animals consisted initially of 8 beagle dogs, 4 rhesus monkeys, 50 Wistar rats, and 40 ICR mice. All animals except rats are female. Exposures are conducted on a 6 hour/day, 5 day/week basis scheduled to cover a 6-month period. As of this writing, 18 weeks have elapsed since the initiation of these studies; this corresponds to approximately 90 days of exposure.

The domes are operated at 725 mm Hg pressure to avoid leakage of MMH, with nominal air flows of 40 cfm. Continuous monitoring of MMH concentrations is performed with an Auto Analyzer (reference 13).

Of the various parameters selected to measure the chronic toxicity of MMH, a significant number have shown positive indications of toxic stress thus far.

Relaxation of the nictitating membrane of a number of dogs exposed to the 5 ppm MMH level was observed as early as 2 weeks after the initiation of this study. This effect has continued since that time. It appears to be minimal and in some cases absent following weekends of no exposure, but increases in severity following 4 or 5 daily 6-hour exposures. The mechanism of this effect is not clear. The animals are decidedly photophobic and show abnormal tearing as well as blinking. Careful examination of the palpebral and ocular conjunctiva of representative test and control dogs will be made later in the experiment.

Mice exposed to 5 ppm MMH have shown some signs of stress. The fur appears rough and yellowed and the animals appear lethargic from time to time. Monkeys and rats have not shown physical signs of toxic effect.

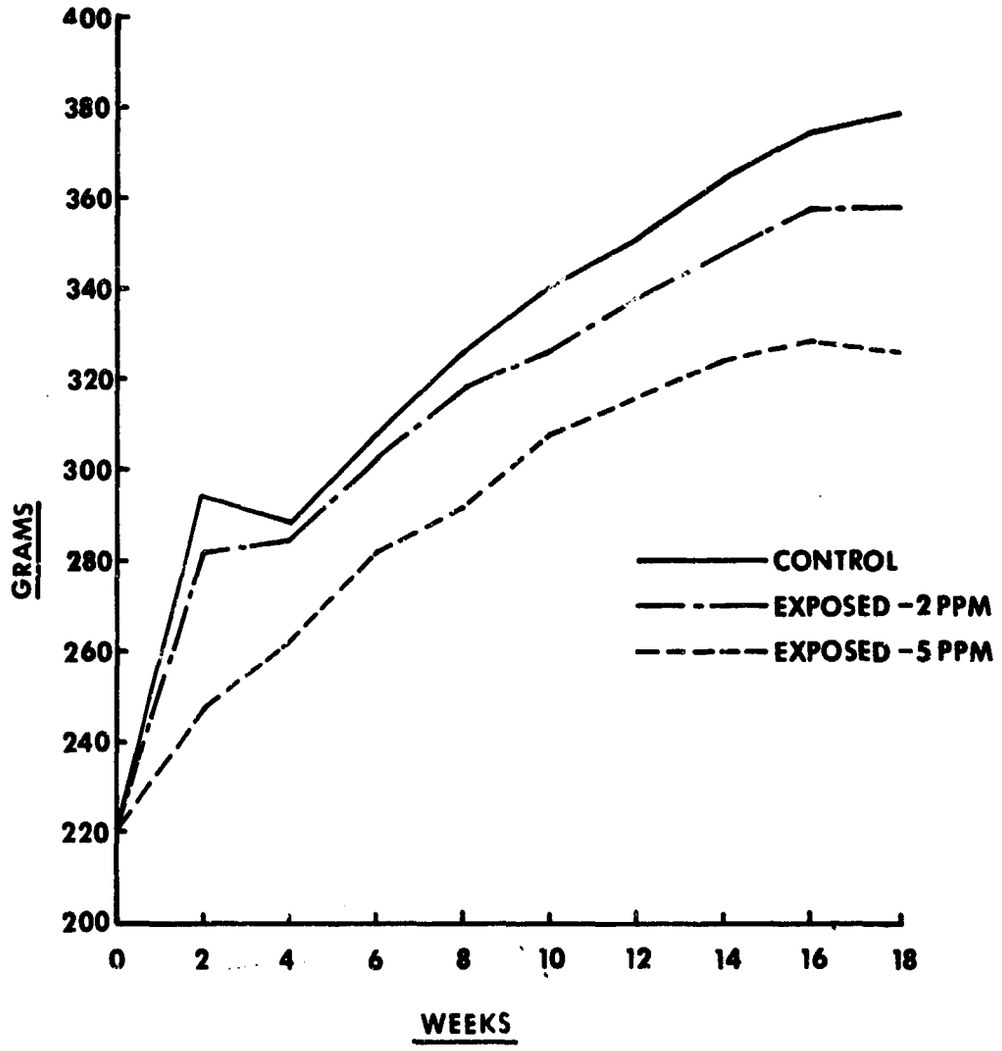
Thus far, deaths have occurred only in mice. Nine, six and one deaths have been recorded respectively for the 5 ppm MMH exposed, the 2 ppm MMH exposed, and the control groups. In the case of the 5 ppm MMH exposure group, seven additional mice died of accidental causes and are not included in the mortality figures. Thus, the corresponding adjusted mortality percentages are 27% for the 5 ppm MMH exposure group, 15% for the 2 ppm MMH exposure group, and 2.5% in the control group, showing a dose related effect. Postmortem gross and histological examinations of the dead mice failed to reveal the exact cause of death. Chronic murine pneumonia was an infrequent finding.

The growth rates of the 3 rat groups are shown in figure 28. These measurements, made at biweekly intervals, illustrate the definite dose dependent effects seen in rodents from chronic exposure to MMH. The 5 ppm MMH exposed groups of rats exhibited statistically significant differences from the control group after 2 weeks exposure. The 2 ppm exposed group of rats, while apparently growing slower by the second week, were statistically different (at the 0.01 significance level) by the tenth week of exposure and remain significantly depressed as of the third month of exposure.

A routine battery of clinical laboratory tests was made on blood samples taken from all large animals prior to the initiation of the experiment in order to establish baselines, then on a biweekly schedule thereafter. The group mean values for hematocrit, hemoglobin, red blood cell and reticulocyte values for all groups of exposed and control monkeys and dogs are graphically presented in figures 29 through 36. Thus far, the hemolytic effects of MMH inhalation are most noticeable in dogs at both the 5 and 2 ppm exposure levels.

The maximum hemolytic response in dogs was seen at the 5 ppm exposure level by the second week and by the fourth week at the 2 ppm level. This profound hemolytic response (50-69% decrease in RBC, HCT and HGB) was accompanied by increased reticulocytosis which continued beyond the time of maximum hemolytic response at the 5 ppm MMH exposure level, but essentially equilibrated at the point of maximum response of dogs to the 2 ppm exposure level. After 4 weeks exposure to 5 ppm MMH the dogs hematologic profile appears to stabilize, although reticulocytosis continued to increase until the tenth week. The stabilization point for the dogs exposed to 2 ppm occurred 2 weeks later, from which point on the level of hemolytic destruction appeared to equal the production rate of new cells. The overall net effect is an approximate 15% reduction in RBC, HCT and HGB levels from preexposure levels.

The hematological measurements of blood taken from monkeys on the same time schedule show this species to be far less sensitive than dogs to the hemolytic effects induced by repeated exposure to MMH.



EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON ALBINO RAT GROWTH

Figure 28

Effect of Chronic Monomethylhydrazine Exposure on Albino Rat Growth

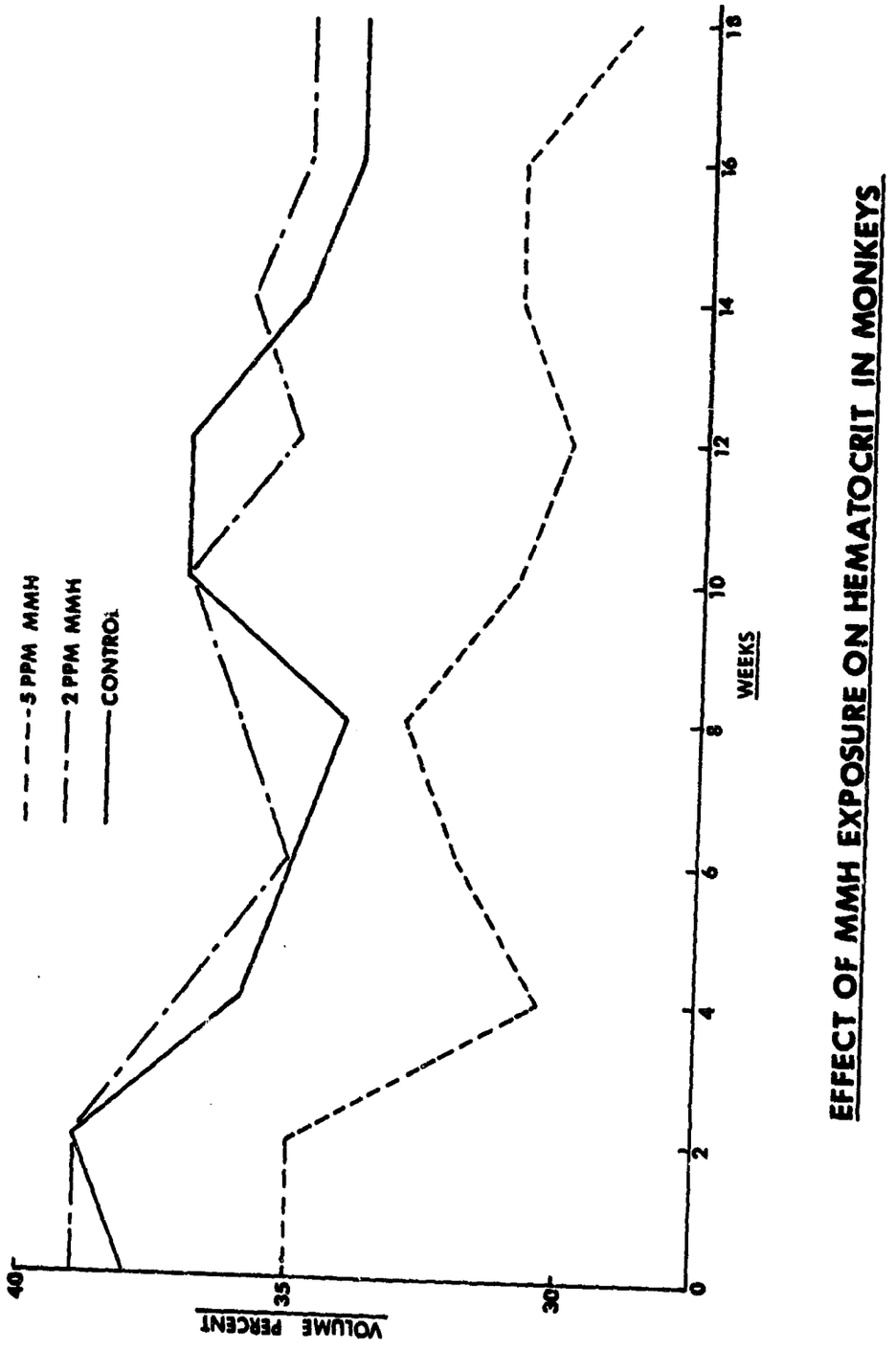


Figure 29

Effect of MMH Exposure on Hematocrit in Monkeys

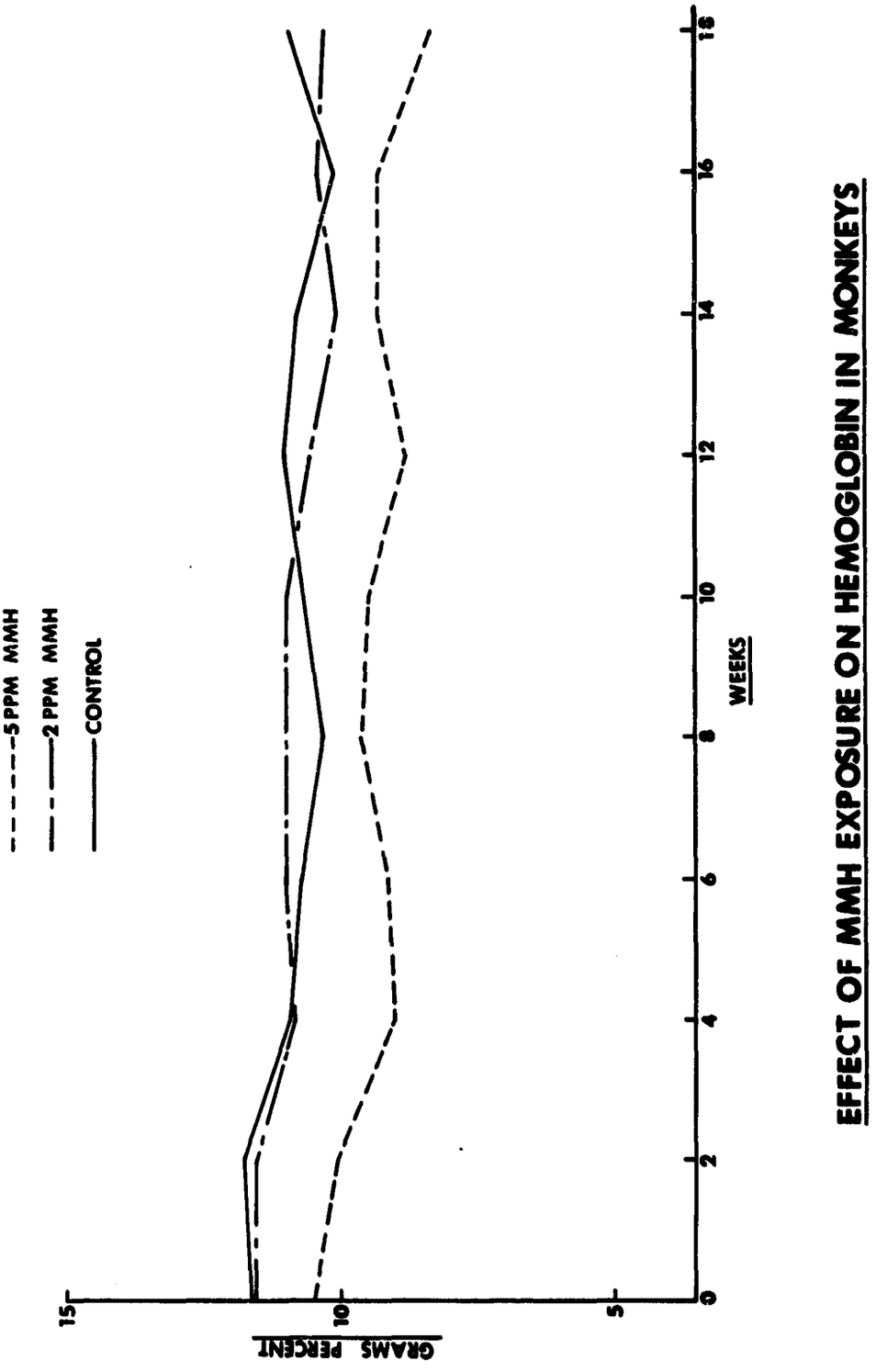


Figure 30

Effect of MMH Exposure on Hemoglobin in Monkeys

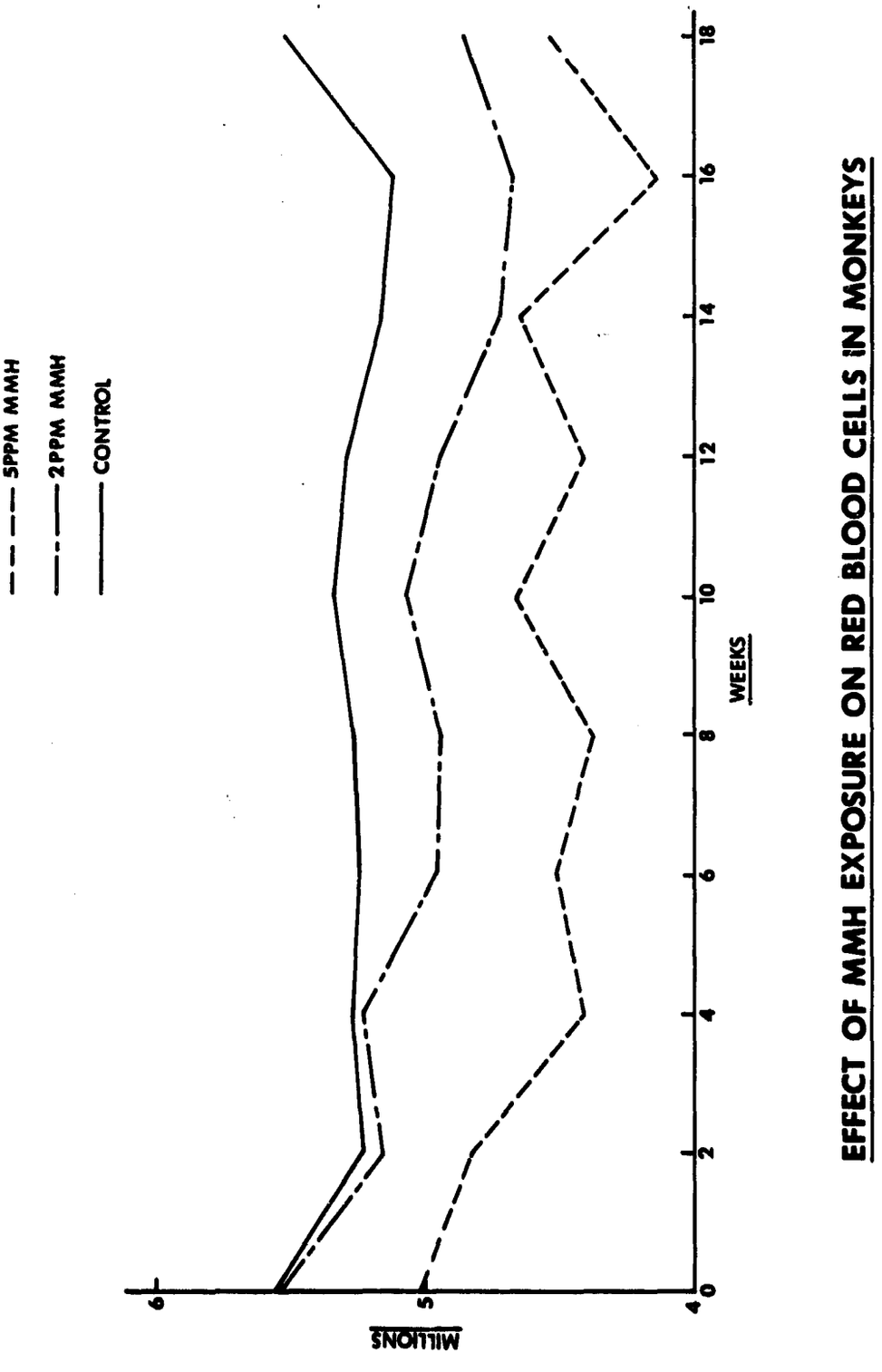


Figure 31

Effect of MMH Exposure on Red Blood Cells in Monkeys

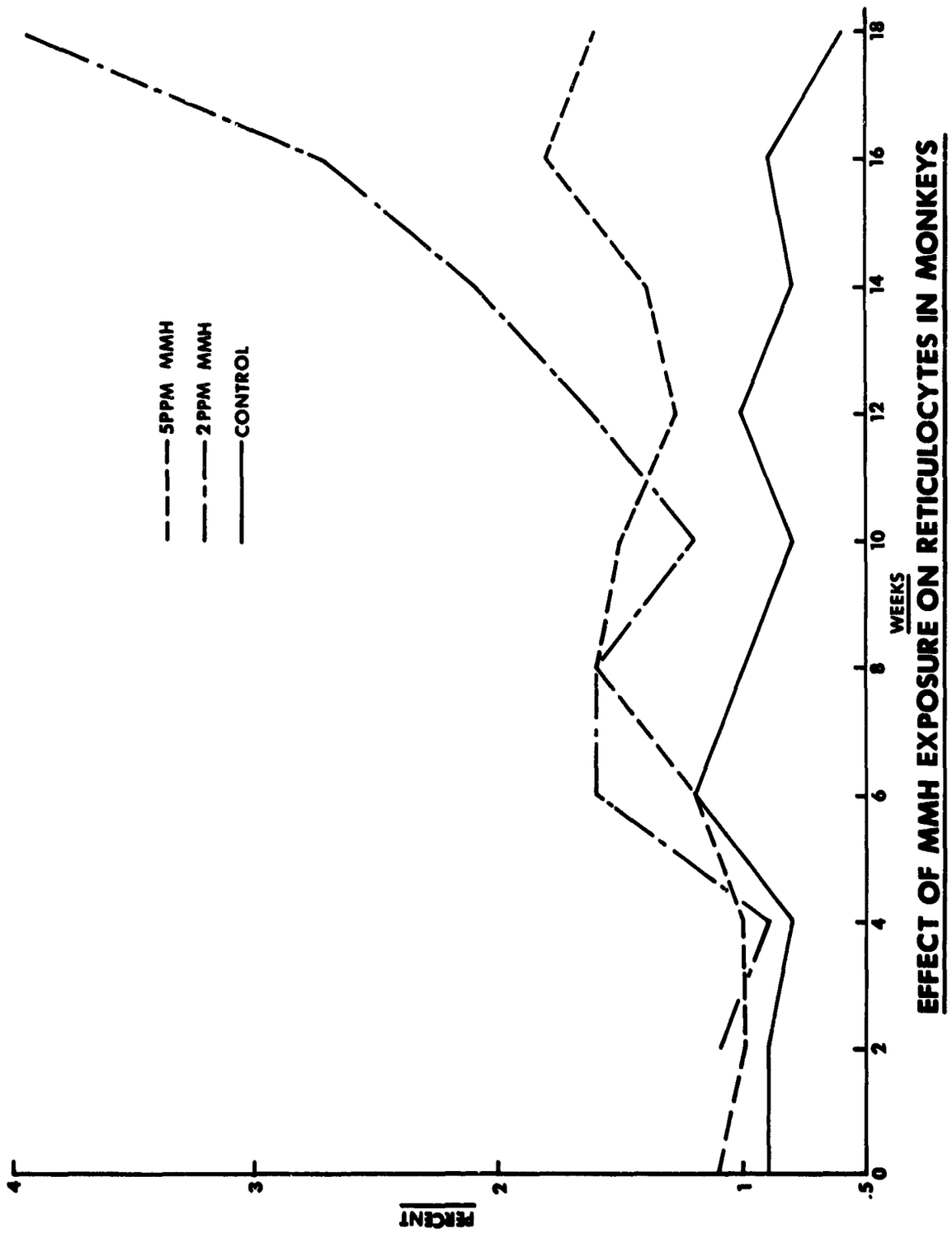


Figure 32

Effect of MMH Exposure on Reticulocytes in Monkeys

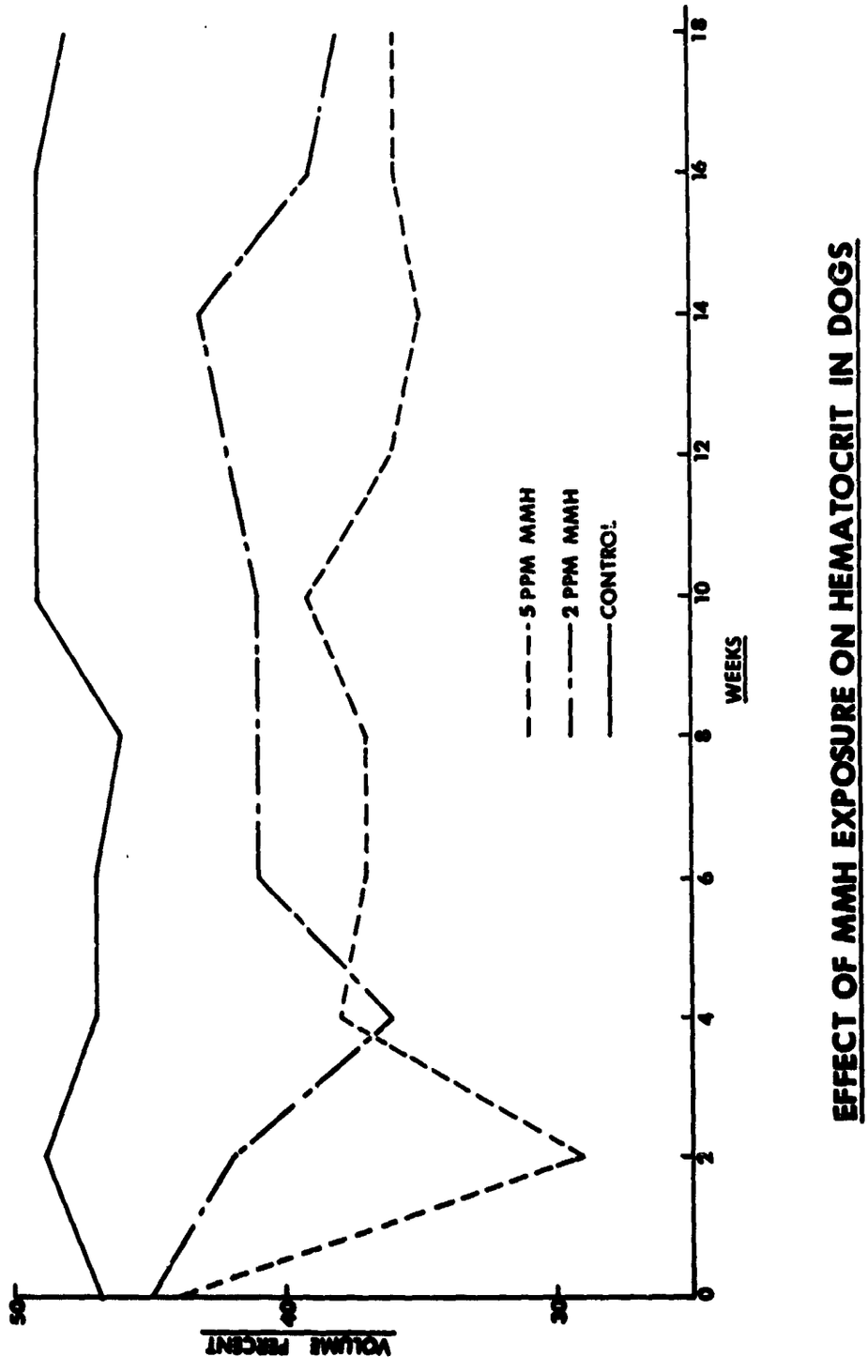


Figure 33

Effect of MMH Exposure on Hematocrit in Dogs

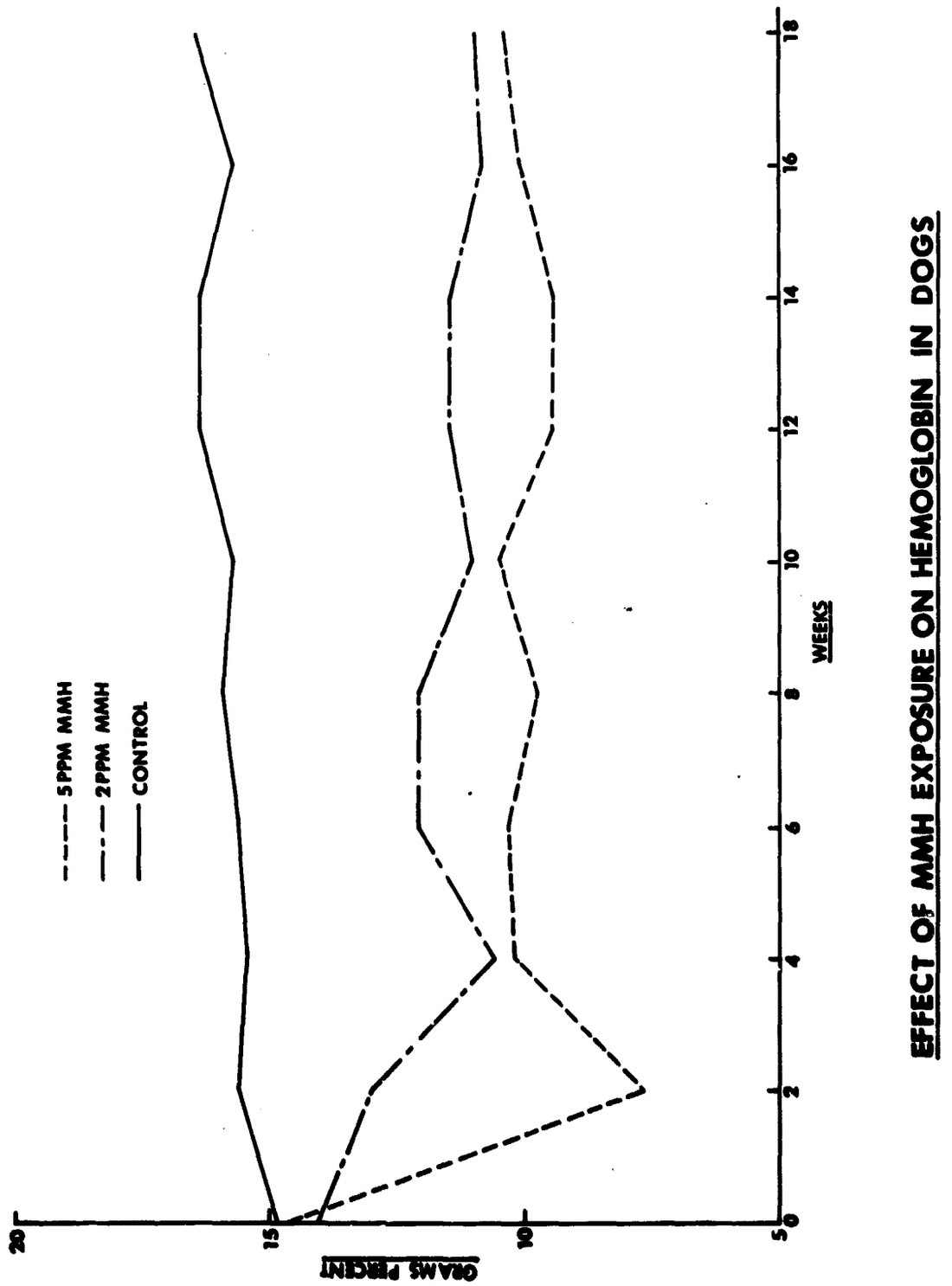


Figure 34

Effect of MMH Exposure on Hemoglobin in Dogs

Figure 35

Effect of MMH Exposure on Red Blood Cells in Dogs

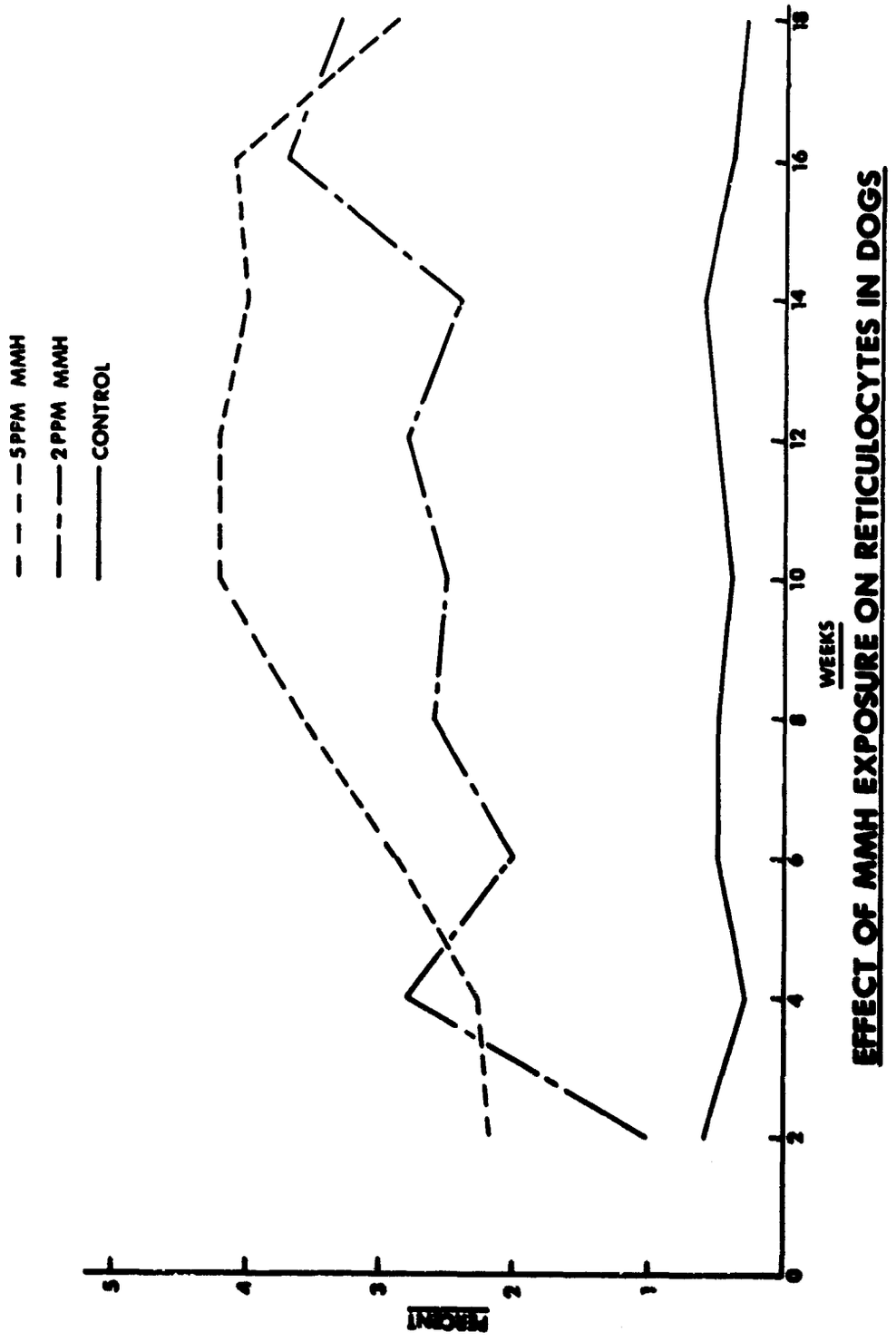


Figure 36

Effect of MMH Exposure on Reticulocytes in Dogs

At 4 weeks, HCT, HGB and RBC values for the 5 ppm MMH exposed monkeys were significantly different, at the 0.05 level, when compared with control animal measurements. A modest rise in reticulocyte counts after 8 weeks exposure was enough to produce statistical differences between test and control values. The net depression of these hematology measurements was 15% below preexposure values.

The rather noticeable rise in reticulocyte counts for monkeys exposed to the 2 ppm level between the tenth and eighteenth weeks was caused by the abnormally high reticulocyte values recorded for one monkey. This animal was surgically fitted with implant electrodes to allow for EEG measurement during the course of the experiment. Subsequent infection, although not endangering life, was enough to adversely influence the mean reticulocyte values recorded for this group. There were no other statistically significant differences at any time interval between the 2 ppm exposed and control monkeys.

The quantitative differences in methemoglobin production between test and control dogs are shown in figure 37. The mean (MHGB) values recorded for dogs exposed to 5 ppm MMH are significantly elevated above those of the 2 ppm exposed and control animals throughout the course of the study. Similarly, but to a lesser extent, the MHGB values of the 2 ppm exposed dogs reflect positive differences from the control values. However, the 2 ppm MMH results are sometimes borderline in significance. Nevertheless, this graph does portray a reasonably clear dose response relationship.

Although the pattern of MHGB formation in test monkeys is not clear, the appearance of Heinz bodies in the blood of these animals provides evidence that some reaction with monkey hemoglobin is taking place.

Blood samples were collected from experimental dogs on a regular biweekly schedule and examined microscopically for the presence of Heinz bodies. Although a consistent pattern was not seen, Heinz bodies were definitely observed in the blood samples of all test animals during the course of this study. Control blood, by contrast, was negative.

Clinical chemistry data collected during the course of the study were screened and tested for trends of biological and statistical importance.

Examination of statistical data comparing mean values of 2 and 5 ppm MMH exposed monkeys with their controls revealed significant differences in a few cases, at the 0.05 level, for BUN and uric acid. These differences occurred only at the second and twelfth week sampling periods.

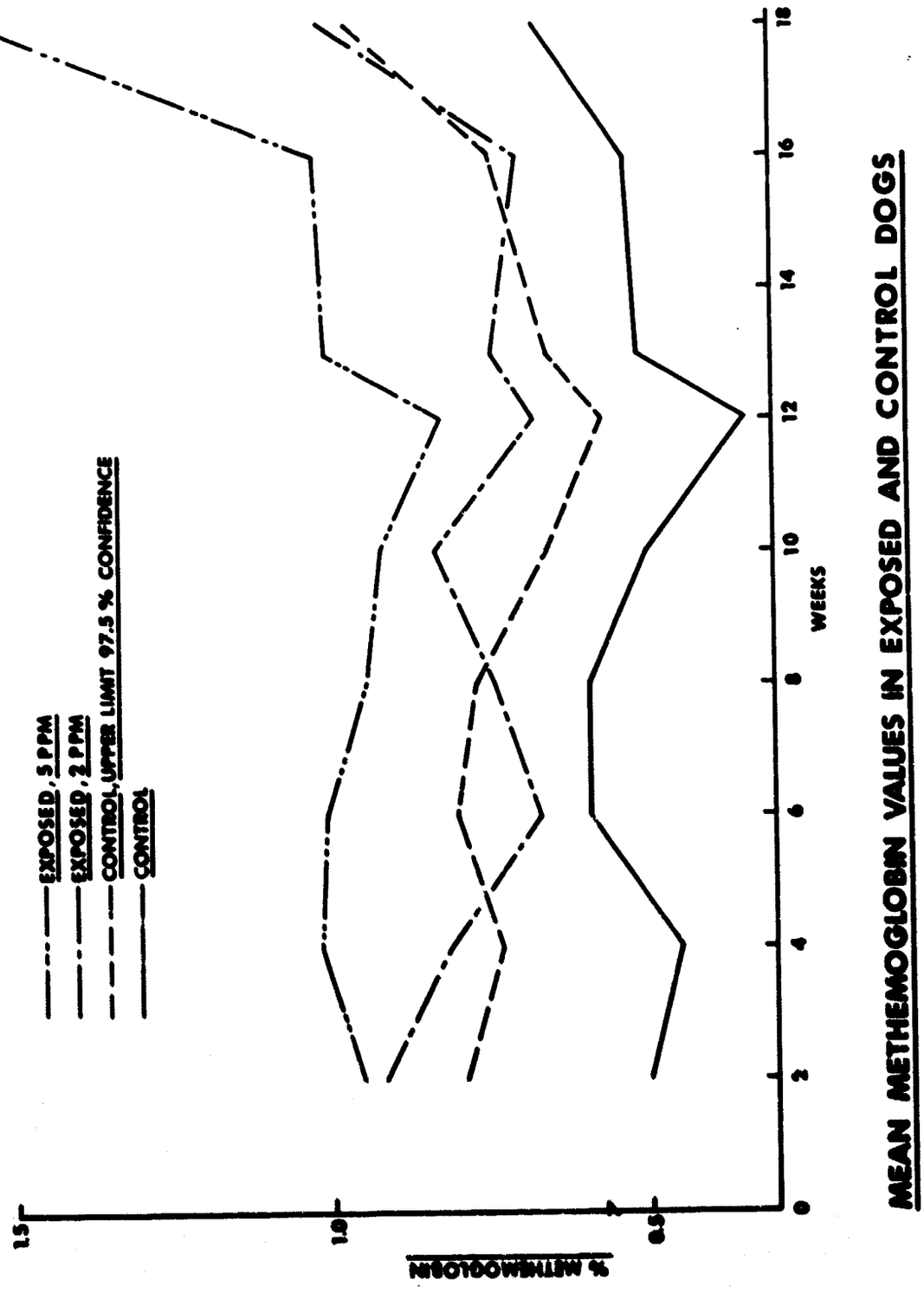


Figure 37

Mean Methemoglobin Values in Exposed and Control Dogs

Both serum bilirubin and alkaline phosphatase levels were significantly elevated in dogs exposed to either MMH concentration from the second week on. The elevation of bilirubin levels is consistent with the previously described pattern of erythrocyte destruction resulting from MMH exposure. The cause of the increase in alkaline phosphatase level has not been established as of this time.

Increased susceptibility of dog red blood cells because of the hemolytic influence of MMH was measured by means of an erythrocyte fragility test. Blood samples collected from one control and two 5 ppm exposed dogs and one control and two 2 ppm exposed dogs were subjected to this examination after 4 and 6 weeks respectively. Results showed that initial hemolysis is noticeably increased when test sets are compared with control samples. For example, a 0.54% salt solution produced 2.6% hemolysis in control dogs blood and 16.7 and 16.5% in the blood samples of two 5 ppm MMH exposed dogs. When the 2 ppm exposed dogs were tested, their comparable values to the 2.6% control animals were 12.5 and 13.6%.

The results of subsequent RBC fragility tests are not yet available. Preliminary information, however, suggests that the trend now is reversing itself apparently because of the production of new red cells which are more resistant to osmotic fragility than the older cell populations.

Previous acute and subacute toxicity tests have shown that CNS damage is caused by exposure to MMH. To study this effect, 3 monkeys, one from each experimental group, were implanted with brain electrodes prior to the start of the study. EEG measurements were made monthly and thus far have been negative in regard to measurable differences between test and control monkeys.

Pairs of rats sacrificed monthly in each dome did not show any differences in gross pathology until the fourth month when pale livers were noted in both the 2 and 5 ppm exposed animals.

These studies are continuing and will be the subject of a subsequent technical report.

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13. ABSTRACT

The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1969 through May 1970 are reviewed in this report. Modification of the animal exposure facilities are discussed including the installation of an automatic weighing system in each Thomas Dome. Acute toxicity experiments were conducted on beta cloth glass fiber dust, chlorinetrifluoride (ClF_3), oxygen difluoride (OF_2), and hydrogen fluoride. Subacute toxicity studies were conducted on 1,1,2-Trichloro 1,2,2-trifluoroethane and methylisobutylketone. The interim results of chronic toxicity experiments on monomethylhydrazine (MMH) are also described.

Key Words: Toxicology
Thomas Domes
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Space Cabin Toxicology
Material Testing
Chlorine Trifluorine
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Hydrogen fluoride
Monomethyl hydrazine
Methytisobutylketone
Trichlorotrifluoromethane

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